



UNIVERSITY
OF TASMANIA

**Reproductive Factors and their Associations with
Osteoporosis and Osteoarthritis in Women**

by

Shuying Wei, MD, BBiotech (Hons)

Menzies Research Institute Tasmania

Submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy

University of Tasmania

July 2012

Statement of Originality

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the Rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

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Statement of Co-Authorship

This thesis includes work which has been submitted for publication in peer-reviewed journals. Shuying Wei (SW) was not the sole author for the publication of the work and was assisted by the co-authors. The contributions of each author are detailed as follows.

Chapter 4: Wei S, Jones G, Thomson R, Dwyer T, & Venn A. Oral contraceptive use and bone mass in women aged 26-36 years. *Osteoporosis International* (2011) 22(1):351-355.

Proportion of contribution:

SW (60%), Jones G (GJ, 10%), Thomson R (RT, 10%), Dwyer T (TD, 5%), Venn A (AV, 15%)

Details of the authors roles:

SW was responsible for data collection, data management and cleaning, undertook all data analyses and interpretation of data, prepared the initial draft of the manuscript, and completed revisions of the manuscript.

GJ and RT participated in analysis and interpretation of data, and critically revised the manuscript.

TD contributed to study design and planning, interpretation of data and critically revised the manuscript.

AV contributed to study design and planning, participated in analysis and interpretation of data, assisted with the initial manuscript draft, and critically revised the manuscript.

Chapter 5: Wei S, Venn A, Ding C, Foley S, Laslett L, & Jones G. The association between oral contraceptive use, bone mineral density and fractures in women aged 50-80 years. *Contraception* (2011) 84(4):357-62

Proportion of contribution:

SW (60%), AV (10%), Ding C (CD, 5%), Foley S (SF, 5%), Laslett L (LL, 5%), GJ (15%)

Details of the authors roles:

SW was responsible for data collection, data management and cleaning, undertook all data analyses and interpretation of data, prepared the initial draft of the manuscript, and completed revisions of the manuscript.

AV participated in analysis and interpretation of data, and critically revised the manuscript.

CD participated in analysis of data, and critically revised the manuscript.

SF and LL participated in data collection and critically revised the manuscript.

GJ contributed to study design and planning, participated in analysis and interpretation of data, assisted with the initial manuscript draft, and critically revised the manuscript.

Chapter 6: Wei S, Jones G, Thomson R, Otahal P, Dwyer T, & Venn A. Menstrual irregularity and bone mass in premenopausal women: cross-sectional associations with testosterone and SHBG. *BMC Musculoskeletal Disorders* (2010) 11:288.

Proportion of contribution:

SW (60%), GJ (10%), TR (5%), Otahal P (PO, 5%), TD (5%), AV (15%)

Details of the authors roles:

SW was responsible for data collection, data management and cleaning, undertook all data analyses and interpretation of data, prepared the initial draft of the manuscript, and completed revisions of the manuscript.

GJ participated in analysis and interpretation of data, and critically revised the manuscript.

RT and PO participated in analysis of data and critically revised the manuscript.

TD contributed to study design and planning, interpretation of data and critically revised the manuscript.

AV contributed to study design and planning, participated in analysis and interpretation of data, assisted with the initial manuscript draft, and critically revised the manuscript.

Chapter 7: Wei S, Venn A, Ding C, Martel-Pelletier J, Pelletier J P, Abram F, Cicuttini F, & Jones G. The associations between parity, other reproductive factors and cartilage in women aged 50-80 years. *Osteoarthritis and cartilage* (2011) 19(11):1307-1313

Proportion of contribution

SW (55%), AV (7.5%), CD (5%) Martel-Pelletier J (JMP, 5%), Pelletier J P (JPP, 5%), Abram F (FA, 5%), Cicuttini F (FC, 5%), GJ (12.5%)

Details of the authors roles:

SW was responsible for data collection, data management and cleaning, undertook all data analyses and interpretation of data, prepared the initial draft of the manuscript, and completed revisions of the manuscript.

AV participated in analysis and interpretation of data, and critically revised the manuscript.

CD participated in analysis and interpretation of data, and critically revised the manuscript.

JMP and JPP participated in the study planning, carried out data collection, and critically revised the manuscript.

FA carried out data collection and critically revised the manuscript.

FC contributed to study design and planning, interpretation of data and critically revised the manuscript.

GJ contributed to study design and planning, participated in analysis and interpretation of data, assisted with the initial manuscript draft, and critically revised the manuscript.

Chapter 8: Wei S, Jones G, Venn A, Cicuttini F, March L, Otahal P, Cross M, Dalton M, Dwyer T, & Ding C. The association between parity and knee cartilage in young women. Manuscript submitted to *Rheumatology*.

Proportion of contribution

SW (55%), GJ (7.5%), AV (5%), FC (5%), March L (LM, 5%), PO (5%), Cross M (MC, 2.5%), Dalton M (MD, 2.5%), TD (2.5%), CD (10%)

Details of the authors roles:

SW was responsible for data collection, data management and cleaning, undertook all data analyses and interpretation of data, prepared the initial draft of the manuscript, and completed revisions of the manuscript.

GJ participated in study design, analysis and interpretation of data, and critically revised the manuscript.

AV participated in study design, interpretation of data, and critically revised the manuscript.

FC and LM participated in study design and critically revised the manuscript.

PO participated in analysis and interpretation of the data, and critically revised the manuscript.

MC and MD participated in data collection and critically revised the manuscript.

TD participated in study design and critically revised the manuscript.

CD contributed to study design and planning, participated in analysis and interpretation of data, assisted with the initial manuscript draft, and critically revised the manuscript.

Signed:Date:

Shuying Wei (Candidate)

Signed:Date:

Alison Venn (Primary Supervisor)

Abstract

Women are at higher risk of both osteoporosis and osteoarthritis (OA) compared with age-matched males. Sex hormones and reproductive factors may partly explain these differences. This study therefore aimed to investigate reproductive factors including parity, menstrual regularity, use of oral contraceptives (OC) and hormone replacement therapy (HRT) and their associations with bone mass, cartilage and radiographic OA in population-based samples of both young and older women.

Young women aged 26 to 36 years were selected from the Childhood Determinants of Adult Health (CDAH) study, a 20-year follow-up of children who participated in 1985 Australian Schools Health and Fitness Survey (ASHFS). Older women aged 50 to 80 years were selected from the Tasmanian Older Adult Cohort (TASOAC) study, an ongoing prospective study in southern Tasmania. Parity, menstrual regularity and use of OC and HRT were assessed by self-administered questionnaire. Bone mass was measured by quantitative ultrasound (QUS) for young women and bone mineral density (BMD) by dual-energy x-ray absorptiometry (DXA) for older women. Knee cartilage volume and cartilage defects were measured by magnetic resonance imaging (MRI) for both young and older women and radiographic OA was assessed by X-ray only for older women.

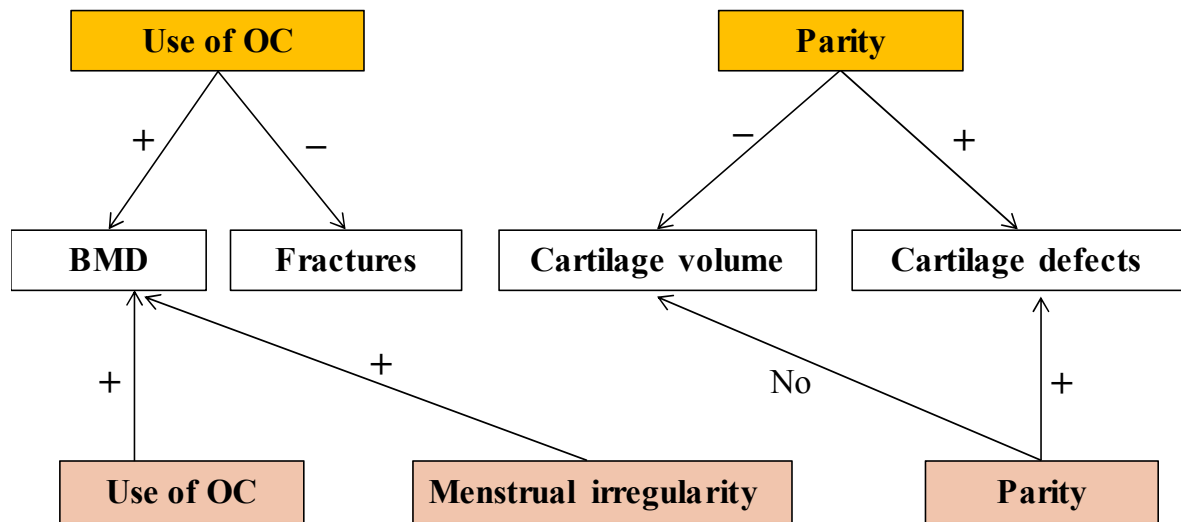
Key findings were:

- Young women:
 - Current use of OC was associated with higher bone mass.
 - Irregular menstrual cycles were associated with higher bone mass and the association was partially mediated by markers of androgen status especially free testosterone.
 - Parity was positively associated with cartilage defects primarily at the patella site. Women with three or more children had the highest prevalence of cartilage defects.

- In older women:
 - Ever use and duration of OC use were associated with higher BMD in the spine and total body measured at age 50-80 years.
 - OC use for five to ten years was associated with a reduction of vertebral fracture.
 - Parity was associated with lower cartilage volume primarily in the tibial compartment and the associations were dose-dependent.
 - Parity was associated with higher cartilage defects only in the patella compartment.
 - There were no associations between parity and osteophytes or joint space narrowing (JSN).
 - Use of OC and HRT was not associated with knee cartilage volume, cartilage defects or radiographic OA including JSN and osteophytes.

In conclusion, these cross-sectional analyses of population-based samples of both young and older women showed use of OC was associated with higher bone mass suggesting a protective effect of OC use on bone health. In young women, menstrual irregularity was associated with alterations of sex hormones but may not be as harmful for bone mass as previously believed. Parity, particularly higher parity, was associated with higher cartilage defects in young women and low cartilage volume in older women indicating an effect of childbearing on the development of OA in women. A diagram below illustrated the main conclusions from this study.

Main conclusions of reproductive factors and their associations with bone mass and cartilage in both young and older women



Pink-face figures indicate factors selected from young women, yellow-face figures indicate factors selected from older women.

Acknowledgements

First and foremost, I would like to express my greatest appreciation to my primary supervisor, Professor Alison Venn. This thesis would not have been accomplished without her guidance, encouragement, and even patient help with writing English. I have been working under her supervision since 2006 while I started doing my Honours. Her enthusiasm and passion for her work, extensive expertise in academic research and warm-hearted personality have deeply impressed me. She has helped not only in my academic development but also in getting the support of a scholarship, providing a work opportunity and financial support for attending a significant international conference. I feel extremely fortunate to have been a PhD student working under her supervision.

I wish to express my gratitude to Professor Graeme Jones, my co-supervisor. His knowledge in both epidemiology and the musculoskeletal area, and intellectual input also made a great contribution to my success. His encouragement and many aspects support have pushed me forward in my academic development. To my statistics supervisor, Dr Russell Thomson, I would like to say thanks very much for his statistical expertise and support in dealing with complex statistical analyses. Although he has been overseas since the second year of my PhD, the statistical skills I learnt under his guidance became a firm basis to overcome the statistical challenges ahead.

Many thanks to Petr Otahal, who became my untitled statistic supervisor since Russell left. He was willing to provide help whenever I approached him. His statistical knowledge has benefited me greatly in understanding some complex statistical terms and concepts, and this has led to a significant improvement in my statistical skills. Great appreciation also to Associate Professor Leigh Blizzard for his statistics expertise and kind help with obtaining my scholarship and travel grant from the University of Tasmania.

I would like to acknowledge the University of Tasmania and Menzies Research Institute for providing me a scholarship over my PhD period. The Childhood Determinants of Adult Health (CDAH) study and the Tasmanian Older Adult Cohort (TASOAC) study were supported by the National Health and Medical Research Council of Australia,

National Heart Foundation, Tasmanian Community Fund, Veolia Environmental Services, Arthritis Foundation of Australia, Masonic Centenary Medical Research Foundation, Royal Hobart Hospital Research Foundation, and University of Tasmania Institutional Research Grants Scheme.

I would also like to acknowledge the CDAH and TASOAC participants for their great involvement and generous contributions of time that made this research possible. To the staff and volunteers, I would like to say many thanks to everyone, in particular Marita Dalton, Catrina Boon and Liz O'Loughlin for their wonderful job in managing these projects.

To fellow PhD students, I wish to thank all of them for their friendship and support, in particular Au Bich Thuy, Bev Curry, Kylie Smith, Fiona Cocker, Dawn Dore, Laura Laslett and Oliver Stannus. Also thanks to academic staff for their welcome and support throughout my PhD period, in particular, Changhai Ding, Seana Gall, Sue Pearson, Kristy Sanderson, Verity Cleland and Tania Winzenberg, who always provided help whenever it was needed.

I dedicate this thesis to my mother to commemorate such a kind woman. She passed away late in the second year of my PhD. She experienced a tough life when I was a young girl but she was always very patient with her children, creating laughter in her family and providing help to the neighbours. Since I moved to Australia, she was always concerned about whether I would get used to the new environment, lifestyle, communicating with English and even the western foods but she never complained that I was always away from her even when she was ill. Thank you, my great Mom.

I would also like to sincerely express my great thanks to my father for his understanding and support, and to my brother and sisters for taking care of my parents in my absence. Finally I would like to express my heartfelt thanks to my husband for his continuous encouragement and support both emotionally and financially which were

behind my achievement. And also thanks to my son for his understanding of my new role of being a full-time student over the PhD period.

Publications Arising from the Thesis

Chapter 4: Wei S, Winzenberg T, Laslett LL, Venn A, & Jones G. Oral Contraceptive Use and Bone. *Current Osteoporosis Report* (2010) 9:6-11

Chapter 4: Wei S, Jones G, Thomson R, Dwyer T, & Venn A. Oral contraceptive use and bone mass in women aged 26-36 years. *Osteoporosis International* (2011) 22 (1):351-355.

Chapter 5: Wei S, Venn A, Ding C, Foley S, Laslett L, & Jones G. The association between oral contraceptive use, bone mineral density and fractures in women aged 50-80 years. *Contraception* (2011) 84 (4):357-362.

Chapter 6: Wei S, Jones G, Thomson R, Otahal P, Dwyer T, & Venn A. Menstrual irregularity and bone mass in premenopausal women: cross-sectional associations with testosterone and SHBG. *BMC Musculoskeletal Disorders* (2010) 11:288.

Chapter 7: Wei S, Venn A, Ding C, Martel-Pelletier J, Pelletier J P, Abram F, Cicuttini F, & Jones G. The associations between parity, other reproductive factors and cartilage in women aged 50-80 years. *Osteoarthritis and cartilage* (2011) 19 (11):1307-1313

Chapter 8: Wei S, Jones G, Venn A, Cicuttini F, March L, Otahal P, Cross M, Dalton M, Dwyer T & Ding C. The association between parity and knee cartilage in young women. Manuscript has been accepted by the *Rheumatology* (ID: KES201).

Scientific Presentations Arising from the Thesis

- 2009** The 2nd Joint Meeting of the International Bone & Mineral Society and the Australian and New Zealand Bone and Mineral Society (ANZBMS)
(Sydney, Australia)
“Menstrual irregularity is associated with higher bone mass in young women through alterations in endogenous androgen” (Poster presentation)
Travel grant awarded from ANZBMS
- 2009** Sharing Excellence in Research Conference. University of Tasmania
(Hobart, Australia)
“Oral contraceptive use and bone mass in young women” (Oral presentation)
- 2010** The Australia and New Zealand Bone and Mineral Society (ANZBMS) 20th
Annual Scientific Meeting
(Adelaide, Australia)
“The association between oral contraceptive use, bone mass and fractures in both young and older women” (Oral presentation).
Travel grant awarded from ANZBMS
- 2010** Sharing Excellence in Research Conference. University of Tasmania
(Hobart, Australia)
“Menstrual irregularity is associated with higher bone mass in young women which is mediated through alterations in endogenous androgen” (Poster presentation)

2011 International Epidemiological Association (IEA) World Congress of
Epidemiology
(Edinburgh, Scotland)

“The association between oral contraceptive use and bone mass in both young
and older women” (Poster presentation).

Travel grant awarded from the University of Tasmania

List of Abbreviations

ABS	Australia Bureau of Statistics
ACER	Australian Council for Education Research
ASHFS	Australian Schools Health and Fitness Survey
BMI:	Body mass index
BUA	Broadband ultrasound attenuation
BMD	Bone mineral density
BMC	Bone mineral content
CATI	Computer assisted telephone interview
CDAH	Childhood Determinants of Adult Health study
CD	Changhai Ding
CI	Confident interval
CV	Coefficients of variation
DMPA	Depot medroxyprogesterone acetate
DXA	Dual-energy x-ray absorptiometry
EE	Ethinyl estradiol
ERT	Estrogen replacement therapy
FT	Free testosterone
FAI	Free androgen index
FFQ	Food frequency questionnaire
GIS	Geographical Information Systems
HRT	Hormone replacement therapy
ICC	Intra-class correlation coefficient

IVF	In vitro fertilization
IPAQ	International physical activity questionnaire
JSN	Joint space narrowing
LTPA	Leisure time physical activity
MRI	Magnetic resonance imaging
MET	Metabolic Equivalent Task
OC	Oral contraceptive
OA	Osteoarthritis
OR	Odds ratio
PA	Physical activity
PCOS	Polycystic ovary syndrome
PCO	Polycystic ovaries
PRR	Prevalence rate ratio
QUS	Quantitative ultrasound
QUI	Quantitative ultrasound index
RIA	Radioimmunoassay
SHBG	Sex hormone binding-globulin
SD	Standard deviation
SDAC	Survey of Disability, Ageing and Cares
SF	Stella Foley
SOS	Speed of sound
TASOAC	Tasmanian Older Adult Cohort study
UK	United Kingdom

USA	United States of America
WHR	Waist hip ratio
WHO	World Health Organization
WOMAC	Western Ontario McMasters Osteoarthritis index

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Synopsis

Osteoporosis and osteoarthritis (OA) are the two major musculoskeletal conditions affecting older people. These diseases contribute substantially to illness, disability and economic burden in our community. Women are at higher risk of both osteoporosis and osteoarthritis compared with age-matched males. The mechanism underlying the gender differences is not fully understood but reproductive factors may be important explanatory factors. This thesis therefore investigated reproductive factors and their associations with bone mass, cartilage and radiographic OA in population-based samples of both young and older women. In this synopsis, an overview of the content of each chapter is presented.

Chapter 1 provides a general introduction to osteoporosis and OA including definitions, risk factors, prevalence, cost and disease burden, and treatments and management of these diseases. In addition, a brief description of evidence linking reproductive factors to osteoporosis and OA is presented.

Chapter 2 lists the research questions to be addressed in this thesis.

Chapter 3 describes the three studies utilized in this thesis including the Childhood Determinants of Adult Health (CDAH) study, the Tasmanian Older Adult Cohort (TASOAC) study and the CDAH Knee Cartilage Study. Descriptions of each study include study population and design, measurements of outcomes, study factors and covariates pertinent to the research questions.

Chapter 4 describes the association between use of combined oral contraceptives (OC) and bone mass in 687 CDAH participants (aged 26-36 years). Contraceptive use was assessed by self-administered questionnaire as OC users, progestogen-only contraceptive users and non-users of hormonal contraceptives. Bone mass was measured by quantitative ultrasound (QUS) including broadband ultrasound attenuation (BUA), speed of sound (SOS) and quantitative ultrasound index (QUI). In multivariable analysis, use of OC was significantly associated with higher bone mass measured as BUA and QUI (all $p < 0.05$), and a similar trend for SOS ($p = 0.06$). Progestogen-only contraceptive users also had higher

BUA than non-users, but the differences were not statistically significant in this small group (n=43). In conclusion, use of OC was associated with higher bone mass measured by QUS in this population-based sample of young women while progestogen-only contraceptives appeared to have no deleterious effect on bone mass.

Chapter 5 reveals the relationships between use of OC, BMD and fractures in 491 TASOAC participants (aged 50-80 years, 88% postmenopausal women). Use of OC and fractures were assessed by self-administered questionnaire; BMD and vertebral fracture by dual-energy X-ray absorptiometry (DXA). OC use was associated with higher BMD at the total body (6%, $p<0.05$) and spine (4%, $p=0.05$) but not hip after adjustment for confounders. There was also a significant association between duration of OC use and total body and spine BMD. Use of OCs for 5-10 years was associated with reduced vertebral fracture (adjusted $p<0.05$) but there was no significant association between use of OC and non-vertebral fractures. In conclusion, OC use and duration of use were associated with higher total body and spine BMD and a consistent reduction in vertebral fractures although most associations did not reach significance.

Chapter 6 presents the associations between menstrual irregularity, hormonal factors and bone mass in 382 CDAH participants. Menstrual regularity was measured by questionnaire and bone mass by QUS (including BUA, SOS and QUI). Androgen status was determined by levels of serum testosterone, sex hormone-binding globulin (SHBG) and the free androgen index (FAI). Menstrual irregularity was positively associated with SOS and QUI (all adjusted $p<0.05$) and a consistent trend for BUA (adjusted $p=0.06$). After further adjustment for hormonal factors (either testosterone, SHBG or FAI), the strength of the associations was moderately attenuated. However, women with irregular cycles still had a 6% higher bone mass measured by QUS. In conclusion, menstrual irregularity was associated with higher bone mass in this population-based sample of young women and the association was partially mediated by SHBG and FAI.

Chapter 7 examines associations of parity and use of HRT and OC with cartilage volume, cartilage defects and radiographic OA in 489 TASOAC participants. Parity, use of

HRT and OC was assessed by questionnaire; knee cartilage volume and the defects by MRI and joint space narrowing (JSN) and osteophytes by X-ray. In multivariable analysis, parity was associated with a deficit in total knee cartilage volume (adjusted $p < 0.05$). Increasing parity was associated with decreasing cartilage volume in both the tibial compartment and total knee (both P trend < 0.05). Parity was also associated with greater cartilage defects in the patella compartment but not other sites. There was a consistent but non-significant increase in knee JSN and osteophytes for parous women. Use of HRT and/or OC was not associated with cartilage volume, cartilage defects or radiographic change. In conclusion, parity is independently associated with lower cartilage volume primarily in the tibial compartment and higher cartilage defects in the patella compartment in this population-based sample of older women.

Chapter 8 describes the associations between parity, cartilage volume and cartilage defects in 144 CDAH Knee Cartilage Study participants (aged 31-41 years). Parity was assessed by questionnaire; cartilage volume and cartilage defects by MRI. Multivariable analyses showed that parity was positively associated with cartilage defects at the patellar ($p < 0.05$), but not tibial sites after adjustment for confounders. Similarly, increasing parity was associated with greater risk of cartilage defects (adjusted p for trend < 0.05). There were no significant associations between parity (as either a continuous or a categorical variable) and cartilage volume measured at patella-tibial or any specific individual site before and after adjustment for confounders. In conclusion, parity was associated with knee cartilage defects primarily at the patellar site in this sample of young women suggesting an adverse effect of parity on cartilage.

Chapter 9 summarizes the findings from this thesis and also suggests directions for future research.

Chapter 1

The Epidemiology of Osteoporosis and Osteoarthritis

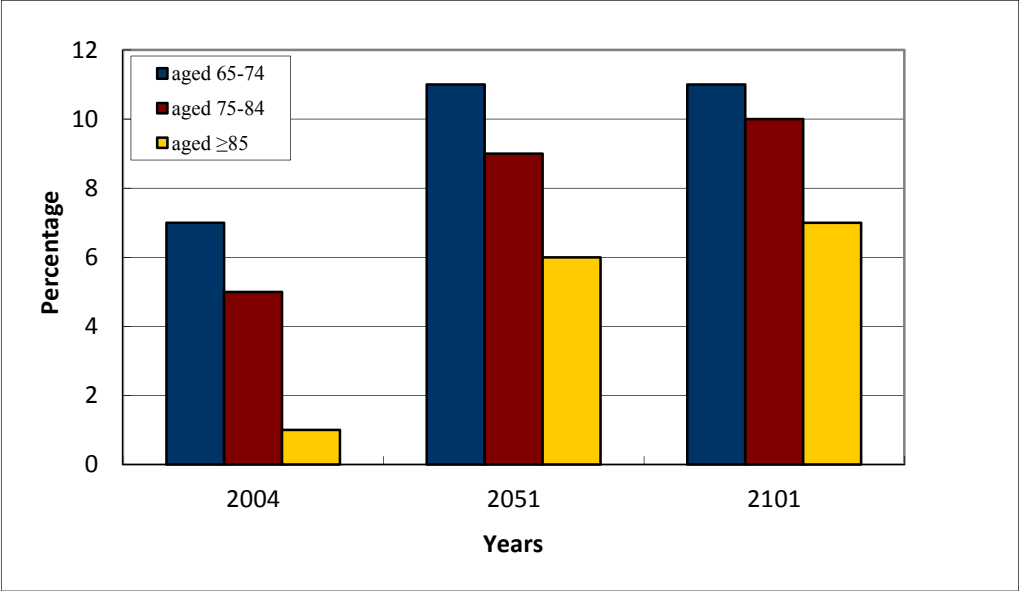
Prelude

In this chapter, osteoporosis and osteoarthritis (OA) which are predominantly diagnoses of older age will be introduced separately including definitions, risk factors, prevalence, costs and disease burden, treatments and management. A detailed literature review regarding each specific study question will be presented in the beginning of each relevant chapter.

1.1 Ageing of the Australian population

The Australian population is ageing and this trend will continue due to a low level of fertility and longer life expectancy (Figure 1). In 2004, about 20% of the Australian population (4.0 million) were under the age of 15 years but this proportion is projected to decrease to between 13% and 16% (series C and A respectively) by 2051, and remaining fairly stable to 2101 (ABS 2005 Population Projections). The population aged 65 years and over, however, will increase rapidly in numbers and proportions of the population. It was estimated that about 13% of the population (2.6 million) were aged 65 and over in 2004, and the percentage is projected to increase to between 26% and 38% (series B and C respectively) by 2051 and to between 27% and 31% (Series B and C) by 2101. As our population ages, the prevalence of age related chronic diseases, in particular osteoporosis and osteoarthritis will significantly increase. These diseases and related medical conditions impose huge economic costs on individuals and society that apply to prevention strategies, medications, and maintenance of independence and quality of life [1, 2].

Figure 1.1. Estimate percentage of Australian population by age and years



Adapted from Australian Bureau of Statistics, Population Projections Series B, Australia, 2004-2101

1.2 Osteoporosis and fractures

Osteoporosis is a disease in which there is a decrease in bone mass and a decrease in skeletal strength with a resultant increased likelihood of fracture. This disease is an important cause of disability, morbidity and mortality, and therefore described as a major public health problem. Osteoporosis is referring to a non-symptomatic disease as it usually causes no symptoms until a fracture occurs most often with minimal trauma. The fractures occur most commonly in the hip, spine and wrist. Osteoporosis and the related fractures are largely preventable, or can at least to be delayed in timing of occurrence [2].

1.2.1 *Definition of osteoporosis*

Osteoporosis is defined as a skeletal disorder characterized by compromised bone strength which result in an increased risk of fracture (Consensus Osteoporosis, JAMA, 2001). Bone strength primarily reflects the integration of bone mineral density (BMD) and bone quality. BMD is determined by peak bone mass and amount of bone loss whereas bone quality refers to architecture, turnover damage accumulation (eg, microfractures), and mineralization. Currently, there is no accurate measure of overall bone strength and BMD is commonly used as a proxy measure of bone strength (Consensus Osteoporosis, JAMA, 2001). Dual-energy X-ray Absorptiometry (DXA) is the current gold standard for measurement of BMD. The DXA scan result is expressed as a “T-score” which represents the number of standard deviations (SD) from the average BMD of a young person (between the age of 20 and 30 years) of the same sex. Osteoporosis is defined if a bone density T-score is at or below 2.5 SD below normal peak values for young adults according to the WHO definition of osteoporosis. Osteopenia, a stage before the occurrence of osteoporosis, is defined as a T-score between minus 1.0 to minus 2.5 SD below the young normal BMD. It can also be defined as a fracture due to minimal trauma regardless of BMD.

1.2.2 *Risk factors for osteoporosis and fractures*

Factors which affect either peak bone mass acquisition in early life or bone loss later in life can be considered risk factors for osteoporosis. While some risk factors are

fixed or unable to be modified such as age, female gender, Caucasian or Asian race, family history of osteoporosis and personal history of fracture, a number of other factors are modifiable via changing lifestyle behaviours including lack of physical activity, cigarette smoking, excessive alcohol consumption, diet low in calcium, vitamin D deficiency and low body mass index.

Some chronic diseases can affect bone mass, such as malabsorption, rheumatoid arthritis, liver diseases, hyperthyroidism, hyperparathyroidism, immobility, such as after a stroke, or from any condition that interferes with walking. These diseases impact bone mass through influences on body metabolism, endogenous hormones or lifestyle and should be considered as risk factors for osteoporosis. Certain medications can also affect bone metabolism such as chemotherapy or long-term use of oral corticosteroids and result in a lower bone mass.

Table 1.1 summarizes key risk factors for osteoporosis by modifiable status [2].

Table 1.1. Risk factors for osteoporosis

Modifiable risk factors	Non-modifiable risk factors
Insufficient physical activity	Increasing age
Low calcium intake	Female sex
Mild vitamin D deficiency	Family history (genetics)
Smoking	Physical disability
Excessive alcohol consumption	A history of fracture
Low body mass index	Some medical conditions and medications

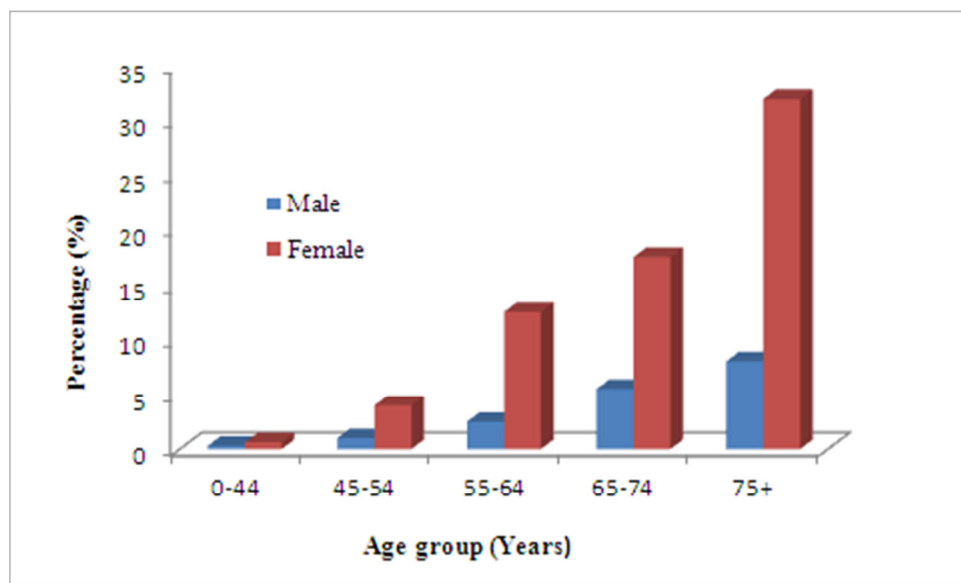
Adapted from AIHW, A picture of osteoporosis in Australia 2008

1.2.3 *Prevalence of osteoporosis*

Osteoporosis is a degenerative process and affects all individuals. However this disease is uncommon before the age of 55 years and significantly increases afterward with

much more females than males appearing in any affected age group [3] (Figure 1.2). The prevalence of osteoporosis is increasing over time as the population ages. In 2001, there were 1.9 million Australians with osteoporosis based on self-reports of having had a doctor-diagnosed osteoporosis but this number increased to 2.2 million in 2007 [4]. The prevalence will increase continuously and is projected to be 3 million by 2021 [4]. As osteoporosis is characterized as a non-symptomatic disease the majority (4 out of 5) of sufferers are unaware of the disease [5], the actual prevalence is expected to be much higher than that estimated.

Figure 1.2. Prevalence of osteoporosis by age and sex, 2007-08



Adapted from AIHW analysis of ABS 2007–08 National Health Survey 2011

Based on self-reports of having had a doctor-diagnosed osteoporosis

1.2.4 Cost and disease burden

Osteoporosis places a financial burden on individuals and society. The cost of this disease involves direct cost such as medication, and indirect costs such as forfeited income due to illness. The total financial costs of osteoporosis are currently estimated at \$7.4 billion per year, with \$1.9 billion of direct costs [4]. Among direct costs, the greatest

proportion of expenditure (35.4%) is on pharmaceutical treatment [6]. The costs are expected to increase in future due to the aging population and projected higher prevalence of the disease.

Osteoporosis causes fractures and increases mortality. One in three women and one in five men aged over 50 will suffer osteoporotic fractures and these fractions increase to one in two women and one in four men when aged over 60 [7]. Hip fractures are the most serious and common reason for hospitalization. In Australia, around 64,000 hospital admissions every year are for bone fractures in people aged 55 and above, and hip fractures constitute more than 37% of the total fracture hospital separations. The proportion of hospitalizations due to hip fractures increased to 55% among those aged 85 and over [6]. Hip fracture is also the major cause of osteoporosis related death. Mortality within 12 months of a hip fracture is estimated to be 24% in Australia and an increased risk of death compared to the general population continues for at least another four years [8]. Estimated remaining lifetime fracture risks are 42% - 44% for women aged over 50 and the figure is higher at 56% for women aged over 60 [9-11].

Vertebral fractures are the most common complications of osteoporosis. However two thirds of patients are asymptomatic and do not come to clinical attention [12]. This results in under-diagnosis of vertebral fractures as high as 29.5% in Australia [13]. The risk of further fractures is increased 4 times following the first vertebral fracture.

1.2.5 Treatment and management of osteoporosis

Bone loss starts around age 30 years and this degenerative process increases after the age of 50. The consequence of bone loss weakens bone strength and leads to fractures which can cause significant disability. Therefore the primary aims of treatment and management of osteoporosis are to achieve maximum peak bone mass and prevent bone loss to reduce the risk of fractures. These involve a range of strategies which may include education, medications and lifestyle and behaviour changes.

Education is a crucial strategy to manage osteoporosis as this disease is commonly asymptomatic before a fracture. It aims to provide important information to improve understanding of what the disease is, the disease process, the causes and risk factors for the disease and available methods to manage and treat this disease. This information will help people to be aware of the best time to accumulate peak bone mass, the appropriate time to prevent bone loss and necessary changes in lifestyle and behaviour. Thus people can take steps to prevent and combat this disease.

Several medications are available for the treatment of osteoporosis. The purpose of treatment is to reduce the occurrence of fractures and its related morbidity and mortality. Bisphosphonates such as risedronate and alendronate are known as effective first-line options for vertebral, hip and non-vertebral fracture prevention (Prevent the next fracture- A guide for pharmacists-Osteoporosis Australia). These medications are subsidised by the Pharmaceutical Benefits Scheme (PBS), once a fracture has occurred, and so are commonly used in Australia. They have been shown to inhibit excessive bone resorption and increase BMD therefore successfully reducing the risk of fractures in women with osteoporosis, with a 40 - 70% decrease in numbers of vertebral fractures and 20% decrease in non-vertebral fractures [14]. Strontium ranelate is another first-line agent approved by Australia and listed by the PBS for the prevention of vertebral and hip fractures through reducing bone resorption and increasing bone formation. Ibandronate sodium is a new bisphosphonate available in Australia. It has been demonstrated to reduce the risk of vertebral fractures in postmenopausal women through its antiresorptive effect (Prevent the next fracture- A guide for pharmacists-Osteoporosis Australia). This agent has better bioavailability (once monthly) than bisphosphonate but has the same effect of antiresorption. However this agent is not yet listed by the PBS nor commonly use in Australia so far.

Estrogen therapy alone (ET) or combination with progestogen (HRT) can prevent bone loss and reduce the risk of fracture in postmenopausal osteoporosis [2]. However, hormone therapy increases the risk of breast cancer and cardiovascular disease meaning it is no longer considered a first-line therapy. Supplementations of calcium and vitamin D may be useful in the management of osteoporosis, particularly for those who have

insufficient vitamin D and calcium intake. But these are not considered as first-line agents for people with osteoporotic fractures as there is less evidence of anti-fracture efficacy.

Other strategies for managing osteoporosis refer to lifestyle and behaviour changes including a balanced and healthy diet, stopping smoking, exercise or being physically active, reducing alcohol intake and keeping a healthy weight [2].

1.2.6 *Reproductive factors and osteoporosis*

Women are at higher risk of osteoporosis than males and sex hormones may play a role in the gender differences. A lower estrogen exposure is associated with lower peak bone mass in teenagers [15, 16] and greater bone loss in postmenopausal women [17] whereas use of sex hormones in postmenopausal women has been shown to prevent bone loss and reduce risk of fractures [18]. These findings suggest that change in either endogenous or exogenous sex hormones may affect BMD. Reproductive factors, such as menstrual regularity and use of oral contraceptives (OC) are associated with changes in sex hormones and thus may be associated with change in bone mass. Evidence from current studies suggests that use of OC is associated with change in bone mineral density [19-21] but the nature of the association is not conclusive. It is also not clear whether the effect of OC use on BMD during the reproductive years continues into the postmenopausal years when bone loss accelerates, and if such change in BMD alters the risk of fracture later in life. The association between menstrual regularity and bone mass has been examined in samples of female athletes and oligo/amenorrhea was found to be associated with lower bone mass. However hormonal factors were not examined in these studies. There have been no studies that have investigated the association in population-based samples in which characteristics such as weight or BMI are significantly different from that in athletes. This thesis will examine the associations between these reproductive factors, bone mass and fractures in population-based samples of both young and older women and the results will be presented in Chapters 4 and 5.

1.3 Osteoarthritis

Osteoarthritis (OA) is the most common joint disorder and the leading cause of pain, loss of function and disability in Australia [1]. The structural changes in OA include the loss of articular cartilage, increased subchondral bone thickness and development of new bone (spurs or osteophytes) at the margins of the joints and subchondral bone cysts. Other tissues may also be affected such as the synovium, ligaments and muscles. OA can affect any moveable joint but the joints of the hips, knees, hands and spine are commonly involved. The management of OA and related disability places huge costs on individuals and the community. It is also reported more often by females than males [22].

1.3.1 *Definition of OA*

OA can be defined based on X-ray abnormalities, clinical symptoms or a combination of the two. The main symptoms of OA are pain, stiffness, tenderness, limitation of movement in the affected joint and swelling.

X-ray has been recognized as the gold standard for the indirect assessment of cartilage and the diagnosis of OA. This technique can identify both joint space narrowing (cartilage loss) and osteophytes (spurs). However, this technique has been criticized as insensitive due to its two-dimensional nature, measurement error and semi-quantitative assessment [23]. Furthermore, the radiographic technology transfers three-dimensional anatomy on to a two-dimensional image which can result in overlap of structures and morphological distortion. While OA is commonly defined by X-ray results or/and symptoms of the affected joint, radiological changes are not always accompanied by joint symptoms.

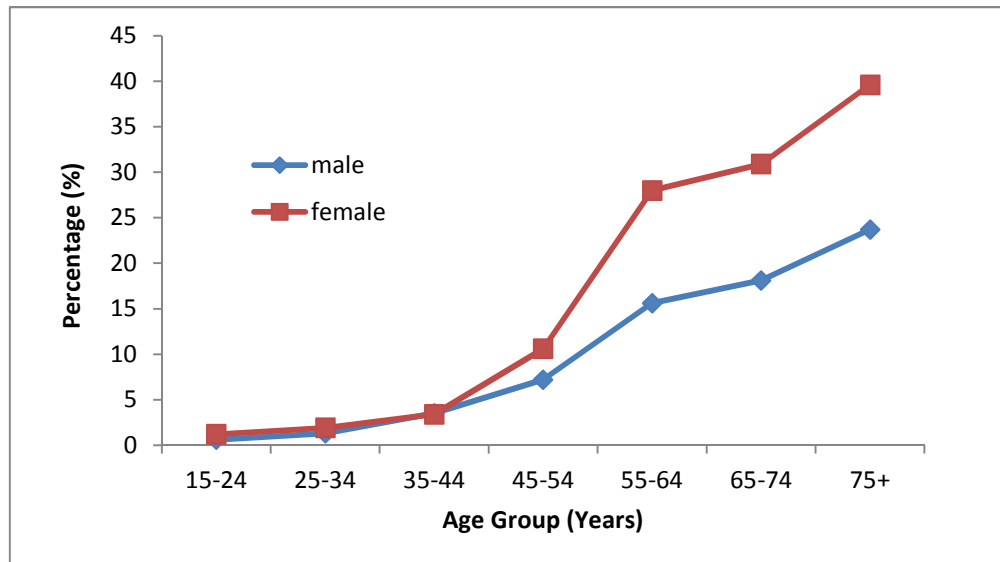
Magnetic resonance imaging (MRI) has been applied to measure cartilage for people with or without OA. This technology can directly visualize knee structure including cartilage volume, cartilage defects and subchondral bone size. It is a sensitive measurement of cartilage loss in longitudinal studies utilizing samples of both OA sufferers and populations without OA [24, 25].

1.3.2 *Risk factors for OA*

Many factors are associated with the alteration of cartilage tissue and factors accelerating cartilage loss are described as risk factors for OA. Risk factors can be considered as either potentially modifiable or un-modifiable. Excess weight or obesity is an important and modifiable risk factor of knee OA, particularly in women [26]. A study has found that the risk of knee OA is increased by 36% for every 5kg of weight increase in middle aged women [27] whereas the risk of knee OA declines by more than 50% if there has been a decrease of same weight during the preceding 10 years [28]. Injury to a joint and overuse of a joint, such as occupational knee bending, have been also recognized as risk factors for knee OA, particularly in men [29], that are potentially modifiable. Un-modifiable risk factors include age, female sex, family history and race such as Asian women [30].

1.3.3 *Prevalence of OA*

OA is the most common arthritis affecting 1.6 million Australian adults in 2007, most of them females [31]. It is degenerative and progressive and the prevalence increases with age, in particular after the age of 45 years. Data from the 2007–08 National Health Survey, based on self-reported history of doctor diagnosed OA, indicate that the prevalence of OA in the 45–54 year age group in females was 10.6% [32]. This proportion increased to 28.0% in the group aged 55–64, and 39.6% at age 75 years and over. A similar trend exists in males but the prevalence is remarkably lower compared with females (Figure 1.3). The prevalence of OA is also expected to increase over time as the population ages. By 2050, it is projected there will be 3.14 million Australians with OA (10.7% of the population), and in females more than males [31]

Figure 1.3. Prevalence of osteoarthritis by age and sex, 2007-08

Adapted from AIHW analysis of ABS 2007–08 National Health Survey 2010

Based on self-reports of having a doctor's diagnosis of OA

1.3.4 Cost and disease burden

OA imposes a large financial burden on individuals and the community. In 2000, the estimated direct health expenditure on OA was \$1,183 million [33]. The major component costs were on hospital services (48%), high-level residential aged care (22%), medication (13%) and out of hospital medical services (11%) [1]. Recent estimates of direct health expenditure on OA was \$2.3 billion in 2007 due to population growth and inflation and the indirect financial costs were more than \$7 billion [31].

OA is the most common cause of disability in Australia. Information from the Survey of Disability, Ageing and Cares (SDAC) conducted by the Australian Bureau of Statistics (ABS) indicates that, in 2003, around 546,000 Australians aged 35 years or over had disability based on self-reported conditions and arthritis is the mainly cause [34]. And these conditions were more often reported by females than males. While there is no national data available on disability specifically associated with types of arthritis, OA would be the major cause of disability as it is the most common type of arthritis. OA

related disability is the third causes of life-years loss in Australian followed by depression and dementia [35].

1.3.5 *Treatment and management of OA*

Five basic principles are suggested for the treatment and management of OA including stop the disease process, keep the joints moving, prevent deformity, reconstruct the joints if needed, and rehabilitate [32]. The purpose of treatment of OA is mainly to reduce symptoms such as pain and to maintain mobility. Joint replacement therapy is commonly used for advanced OA as it reduces pain and disability. This surgery is cost-effective and highly successful for the majority of people with advanced OA. In Australia, 26,712 total knee and 19,279 total hip replacements were performed in the year 2007-2008 [32]. Steroidal anti-inflammatory and non-steroidal anti-inflammatory drugs are commonly used to reduce pain and inflammation, improve mobility and slow disease progression. However there are considerable side effects with these drugs.

Primary prevention of OA is to control weight, maintain fitness and protect joints against damage [32]. Obesity is one of the major risk factors for OA thus management of obesity, such as through weight reduction and maintaining a healthy weight, might reduce the load on the joint and improve joint flexibility that protect joints and reduce pain. However evidence from a randomized trial suggested that weight loss of around 5% had only a modest effect on self-reported pain and physical function as well as objective measures of physical performance in overweight and obese subjects [36]. Other management options include physical therapy and exercise to reduce joint stiffness and increase muscle strength, especially of muscles surrounding the joints.

Secondary preventions refer to adopting a healthy lifestyle including a healthy diet and regular physical activity, and avoiding joint injuries. These measures can prevent or delay the onset of OA and its various complications.

1.3.6 *Reproductive factors and OA*

OA is more common in females than males suggesting that sex hormones or hormonal related reproductive factors may make a contribution to the development and progression of OA. Studies of the use of HRT or estrogen replacement therapy (ERT) and their effects on radiographic OA, however, have given mixed results with protective effects [37], no significant effects [38, 39] and even worsening effects [40-42] being reported. Few studies have investigated OA and the associations with other reproductive factors in particular parity. There have been no studies that have investigated the associations between parity and knee cartilage with assessment of cartilage by MRI which has been shown more sensitive than X-ray. This topic will be addressed in chapters 8 and 9 of this thesis.

Chapter 2

Research Questions

With population-based samples of both young and older women, these questions were addressed in this thesis:

1. Is current use of oral contraceptives (OC) associated with bone mass in young women?
2. Is ever use of OC associated with bone mineral density and fractures measured in later life?
3. Is menstrual regularity associated with hormonal factors and bone mass in young women?
4. Are parity and use of OC and hormone replacement therapy associated with cartilage volume, cartilage defects and radiographic osteoarthritis in older women?
5. Is parity associated with cartilage volume and cartilage defects in young women?

Chapter 3

Methods

Prelude

This thesis used data from three different studies which included the Childhood Determinants Adult Health (CDAH) study, the Tasmanian Older Adult Cohort (TASOAC) study and the CDAH Knee Cartilage study. These studies had a variety of aims, study populations and measurement of outcomes, exposures and other study factors thus the study methods are introduced separately. Descriptions of each study include study population and design, measurements of study outcomes, study factors and other covariates. Exclusion criteria and statistical analyses relevant to each study question will be described in their relevant chapters.

It should be noted that the following chapters are presented in the form in which they were submitted to, or accepted by, peer-reviewed journals for publication. Thus, throughout these chapters there are some differences in the description of methods, analyses, results, and interpretations, due chiefly to requests from journal reviewers.

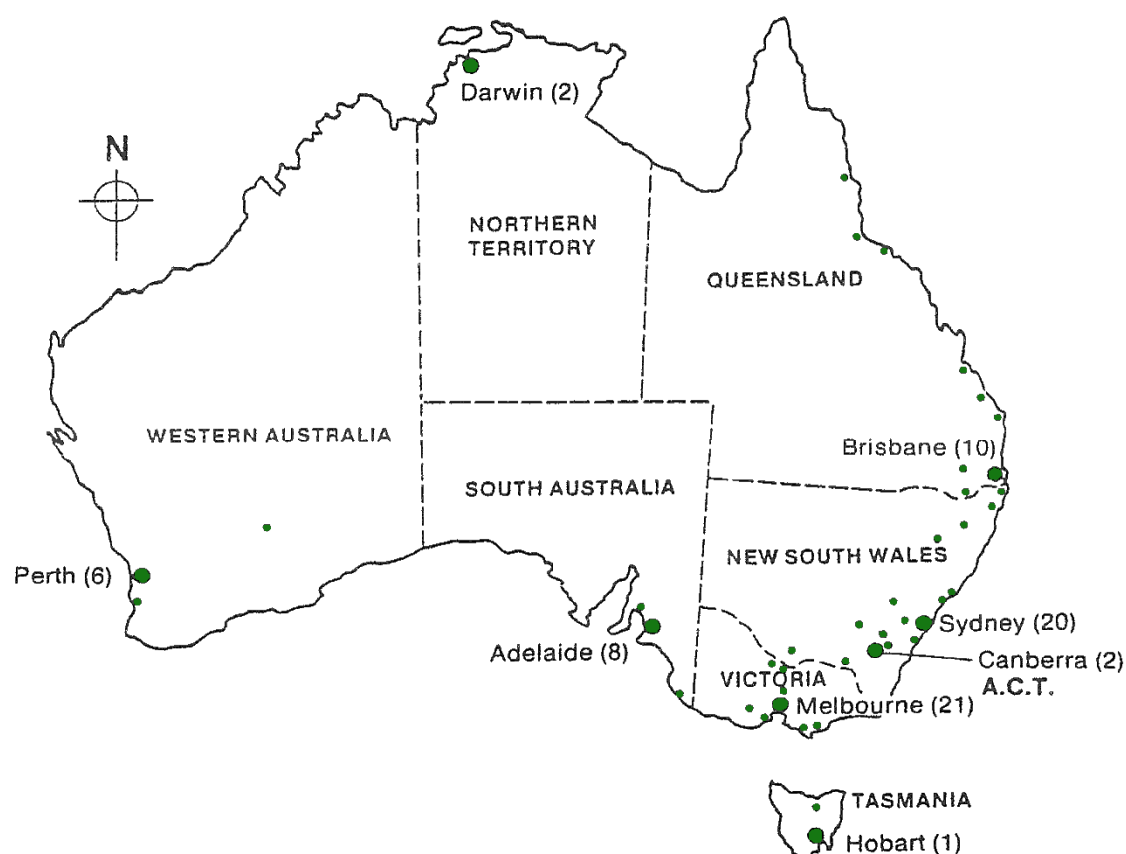
3.1 The Childhood Determinants of Adult Health Study

3.1.1 *Study population and design*

The Childhood Determinants of Adult Health (CDAH) study is a 20-year follow-up of children who participated in the Australian Schools Health and Fitness Survey (ASHFS) in 1985. ASHFS examined the health and fitness of 8498 Australian children aged 7-15 years who were selected randomly from 109 schools Australia-wide. The vast majority of participants were Caucasian Australians. Details of the 1985 sampling strategy have been described elsewhere [43]. Briefly, there were two strategies involved in sample selection of ASHFS. The first stage was to select eligible schools which were able to provide groups of ten students in required age and sex categories. Eligible schools were listed in ascending postcode order to ensure a wide geographical distribution. 109 schools were selected from post code with a random start and constant interval (Figure 3.1) .The Australian Council for Education Research (ACER) conducted the sampling procedure.

Figure 3.1. Distribution of schools surveyed in the ASHFS 1985

Source: Pyke 1985 [44]



The second stage involved the selection of students. Students were categorised by age and sex based on school enrolment information, with 15 students from each age/sex category being systematically selected. Approval was granted to contact schools by the State Directors General of Education, and parental and child consent was required. Among 12,578 students who were aged 7-15 years and invited to participate in the ASHFS, 8498 participated, representing an overall response rate of 67.5%.

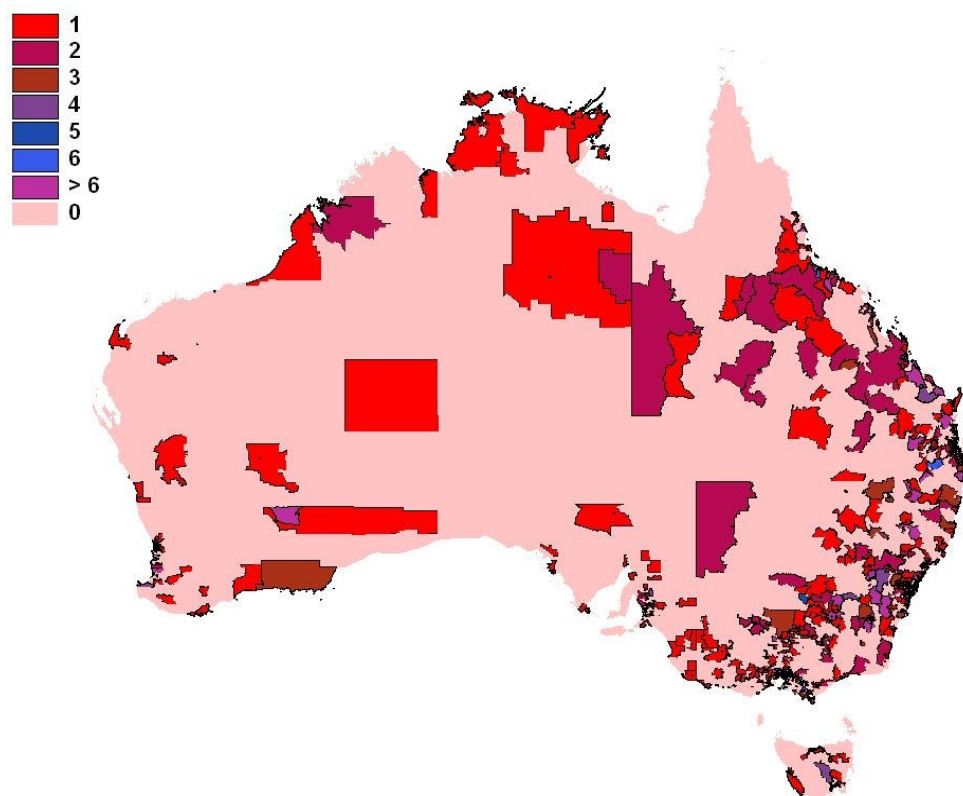
Intensive tracing of participants who participated in 1985 was conducted for the CDAH follow-up study during 2004-2006. A mixed approach was used for tracing participants which included searching current and historical electoral rolls, electronic telephone listings, the National Death Index, and contact with located classmates.

Potential participants were sent an information package which contained an invitation letter (Appendix 1), project information (Appendix 2), a consent form (Appendix 3) and an enrolment questionnaire (Appendix 4). Of the original 8498, 6849 (80.6%) were successfully traced, and 5,170 individuals agreed to participate in the CDAH study and completed an enrolment questionnaire (61% of total sample, 76% of those traced) between 2001 to 2004. A total of 2410 participants (52% female, 48% male) attended one of 34 study clinics in major cities and regional centres around Australia for extensive physical measurements in 2004-6. Anthropometrics, blood biochemistry, lung function, muscle strength and quantitative ultrasound of the heel were measured during the clinic visit and a mental health questionnaire completed. Study clinics were held at local schools, community centres, halls, and other similar venues across Australia. Geographical Information Systems (GIS) software was used to map participants' current postcode (Figure 3.2) in order to determine clinic locations and to facilitate participants' attendance.

In study clinics participants completed a series of measurements, some of which needed to be completed in a specific sequence (Figure 3.3). The measurements of blood pressure, body composition and carotid ultrasound were followed by fasting blood sample collection and breakfast. After breakfast, participants were required to complete a computer-administered mental health questionnaire, lung function test, heel bone density measurement, cardiorespiratory fitness and muscular fitness test, and a pedometer was issued before participants left the clinic.

The aims of the CDAH study were to contribution of childhood factors to the risk of adult cardio-metabolic diseases, mental and musculoskeletal health. All participants provided written informed consent and the study was approved by the Southern Tasmania Health and Medical Human Research Ethics Committee (Ethics Approval Number: H0008152)

Figure 3.2. Distribution of participants across Australia in the CDAH study at follow-up (2004-2006)



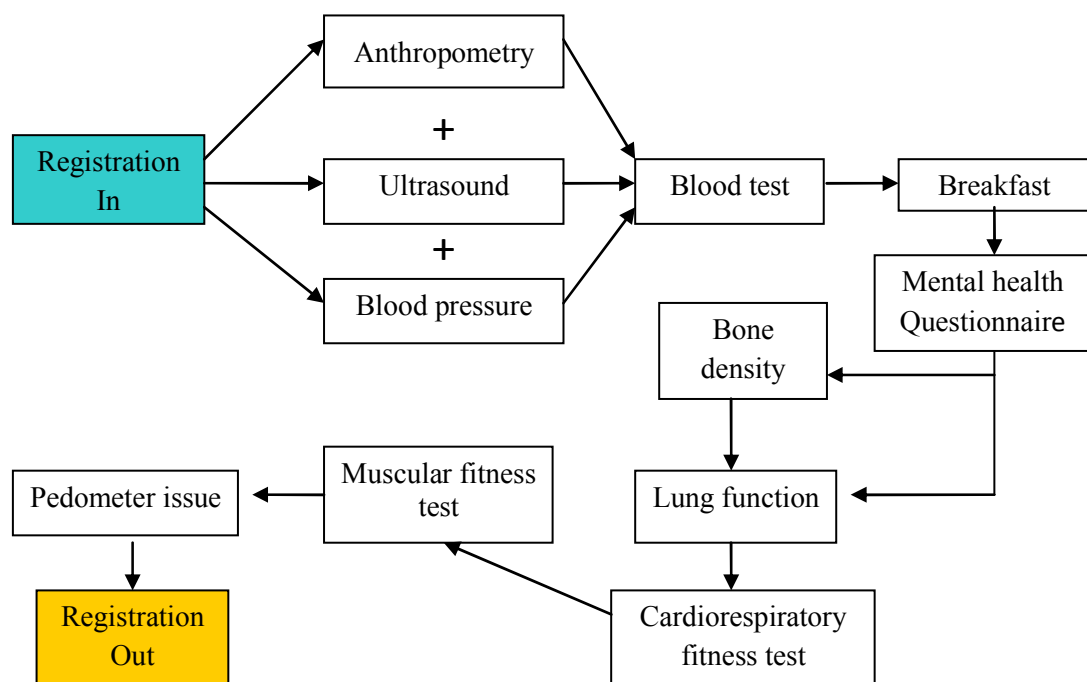
3.1.2 *Measurement of outcomes*

Bone mass

Bone mass was measured by quantitative ultrasound (QUS). QUS has been developed and widely applied in recent years for the measurement of musculoskeletal status and number of diseases associated with bone loss and increased fracture risk. QUS is safe and does not need ionising radiation therefore it has been extensively used in the paediatric setting, as well as in adults, to help clinicians assess bone health in children and monitor their bone growth and development [45-47]. Since it has the advantages of a non-invasive method, low cost and portability, this technology is also widely applied in epidemiological studies for the measurement of bone health, assessment of osteoporosis, and investigation of risk factors for fractures [48-52]. QUS results correlate well with BMD measured by DXA at the heel [53] and measurements of bone mass by QUS were

significantly associated with risk factors for fractures in women in a similar degree to DXA [54]. QUS has been suggested to be a valid alternative to evaluate fracture risk in situations where DXA is not accessible [54].

Figure 3.3. Order of testing during clinic visits in the CDAH follow-up study



In this study, QUS was measured at the heel by using a single Sahara Clinical Bone Sonometer (Hologic Inc., MA, USA) (Figure 3.4). The ultrasound system consists of two sound transducers mounted coaxially on a motorized calliper; one transducer acts as an emitter and the other as a receiver. This makes direct contact with the heel through elastomer pads and an ultrasonic coupling gel. The lower part of the dominant leg was placed immobilized during measurement and the proper leg angle set by a positioning aid. Two parameters, including broadband ultrasound attenuation (BUA) and speed of sound (SOS), were measured by the machine at a fixed region in the mid-calcaneus. BUA was derived from attenuation of the ultrasound waves through the bone and expressed as dB/MHz. SOS was derived from the velocity of the ultrasound and expressed as m/s. The quantitative ultrasound index (QUI) was calculated based on combinations of both SOS

and BUA by using the equation: $QUI = 0.41 \times (BUA + SOS) - 571$. Quality assurance was performed daily by calibrating the device on a dedicated phantom supplied by the manufacturer. The coefficient of variation (CV) for QUS measures was 1% [55].

Figure 3.4. Single Sahara Clinical Bone Sonometer



3.1.3 *Measurement of study factors*

Menstrual regularity

Menstrual regularity was recorded by self-administered questionnaire (Appendix 5). We defined the menstrual cycle as the time from the first day of one period to the first day of the next. Then the question was asked: “thinking about the most recent time when you were having periods and were not using hormonal contraceptives (e.g. the pill) and were not pregnant or breastfeeding, would you describe your periods as very regular, fairly regular, irregular or very irregular” An irregular cycle was defined if women described their menstrual cycles as irregular or very irregular whereas a regular cycle was defined if they answered either very regular or fairly regular. A similar definition of menstrual regularity has been used and validated in a prior study [56].

Current use of oral contraceptives

The information on current use of oral contraceptives (OC) was obtained by the same questionnaire. The question asked “are you currently using any of the following hormonal contraceptives, even if you are using them for reasons other than contraception?” Participants were asked to choose one of nine optional responses which included oral contraceptive pill, minipill (progesterone only pill), weekly contraceptive patch, progestagen (e.g. Implanon), progestagen injection (e.g. Depo provera), progestin injection (e.g. Noristerat), progestin releasing intrauterine device (e.g. Mirena, Copper T380A), progestin releasing implant (e.g. Norplant) and others (please specify) if none listed was applicable. Current use of contraceptive status was then categorized as oral contraceptive users (OC-user) if women were currently using oral contraceptive pills, progestin-only contraceptive users (progestin-user) if they were currently using any type of progestogen-only contraceptives which including oral, injectable and implanted types and progestin releasing intrauterine devices, or non-hormonal contraceptive users if women were not currently using any hormonal contraceptives.

Hormonal factors

Participants had blood samples (32ml) drawn in study clinics for biochemical testing after an overnight fast. Total testosterone and SHBG were measured in female participants who were not using oral contraceptives at a specialized reproductive endocrine laboratory (the Queen Elizabeth Hospital in South Australia). Total testosterone concentrations were estimated by radioimmunoassay (RIA), developed by Repromed Laboratory (Dulwich, South Australia), which is sensitive for lower level of testosterone down to 347pmol/L. The measurement of SHBG was by a non-competitive liquid-phase immunoradiometric assay (SHBG-IRMA kit, Orion Diagnostica, Espoo, Finland). For testosterone, the intra-assay and inter-patient coefficients of variation (CV) were 6% at 1 nmol/L and 15% respectively. For SHBG, the intra-assay and inter-patient CV were 15.4% and 2.0-8.6% respectively. Free androgen index (FAI) was calculated as testosterone (nmol/L) * 100 / SHBG (nmol/L).

Plasma insulin was initially measured by a microparticle enzyme immunoassay kit (AxSYM; Abbot Laboratories, Abbot Park, IL) and later, following a change in the choice of kit by the testing laboratory, by electrochemiluminescence immunoassay (Elecsys Modular Analytics E170; Roche Diagnostics, Mannheim, Switzerland). Due to this change in assay methodology, insulin levels from participants' samples (N = 896) assayed using the first methodology were corrected to levels in samples assayed using the second methodology (as per correction factor equation determined by the laboratory).

3.1.4 *Measurement of covariates*

Parity and other reproductive factors

The information on parity and other reproductive factors was obtained by the questionnaire. These included year when the first period started (age at menarche), history of hysterectomy and whether ovaries were removed, and if they had ever been told by a doctor that they had polycystic ovaries (PCO) or polycystic ovary syndrome (PCOS). In addition, a history of pregnancies was asked by the questions “Have you ever been a pregnant?” and “How many live birth have you had”. Parity was then defined based on the numbers of live births. Women who had at least one live birth were also defined as parous whereas women who had never had a live birth or had never been a pregnant were defined as nulliparous.

Smoking status

Smoking history was assessed by questionnaire. The questions used were: “Have you ever been a regular smoker?” and “Are you currently a regular smoker?” A regular smoker was defined as someone who had smoked at least 7 cigarettes, cigars or pipes every week for at least 3 months. Smoking status was then categorized as a current smoker or non-current smoker which including never smokers and ex-smokers.

Dairy food consumption

Dairy food consumption was assessed by the food frequency questionnaire (FFQ) (Appendix 6) which was a modified version of one previously used in the 1995 National

Nutrition Survey and was based on an existing FFQ developed for Australian populations [57, 58]. The FFQ asked for the average number of times each dairy food was consumed over the previous twelve months. The range of dairy food items included flavoured milk drinks, milk in hot beverages, milk added to breakfast cereal, cream or sour cream, ice-cream, yoghurt, plain or flavoured, cottage or ricotta cheese, cream cheese, and cheddar and other cheeses. Each item had nine optional frequencies rang from "never or less than once a month" to "six or more times per day". Daily serves of dairy items were calculated based on the information obtained.

Alcohol consumption

Alcohol consumption was assessed by FFQ. This questionnaire asked about the frequency of consumption of nine alcoholic beverages (light beer, medium strength beer, full strength beer, red wine, white wine or champagne/sparkling, wine cooler, spirit-based mixed drinks, sherry/port/fortified wines and spirit/liquers), that were consumed in the last twelve months. The average alcohol concentration was then used to estimate the number of standard drinks (10 gram of alcohol) consumed per week, based on the Australian standard drink guide [59]. Responses of never or less than once per month were given a value of zero.

Body composition

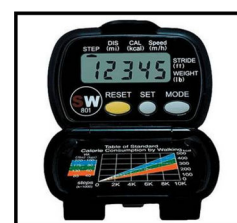
All participants had anthropometric measures taken at the clinics. The measurements were taken three times but if the first two measurements were the same, it was not necessary to take the third measure. Height was measured by Leicester Height Measure (Invicta, Leicester, UK) with shoes removed, and being preferably barefoot. Weight was measured using Heine scales (Heine, Dover, NH, USA) when participants removed any heavy clothing, and any heavy articles from pockets such as keys and wallets. Height was recorded to the nearest millimetre and weight to the nearest 0.1 kg. Waist and hip circumferences were measured using a Lufkin Steel (non-stretch) Tape Measure and recorded to one decimal point. The waist circumference was taken at the level of the narrowest point between the lower costal (10th rib) border and the iliac crest. The hip circumference was taken at the level of the greatest posterior protuberance of the

buttocks. Body mass index (BMI) was calculated as weight (kg) divided by height (cm) in meters squared (kg/cm^2). Waist hip ratio (WHR) was calculated by dividing waist by hip circumference.

Physical activity

Total physical activity was assessed by the self-administered long version of the International Physical Activity Questionnaire (IPAQ-L) [60]. The IPAQ takes account of the frequency, duration and intensity of physical activity that participants performed for duration of at least 10 minutes during the last 7 days. This questionnaire considers a range of physical activities including occupational, commuting, household and yard, and leisure time. Total physical activity was calculated by the equation of Metabolic Equivalent Task (MET) minutes/week based on the time spent over the 7 days and considered all types of physical activities together from the IPAQ-L. In addition to overall time spent in physical activity, leisure time physical activity was calculated separately.

Physical activity was also measured by using pedometers in this study. Participants were asked to wear a Yamax Digiwalker (SW-200) pedometer over the right hip for 7 days and reset the pedometer each day before starting again. A diary (appendix 7) was issued by participants to record the date, the time they commenced wearing the pedometer, the time they took off the pedometer and the total steps recorded for the day. A seven-day period was used to estimate daily steps. The average number of hours of pedometer wearing was calculated from the start and end times recorded by participants. A minimum of 8 hours wear time was required for daily records and a minimum of 4 days reading was required per participant [61]. Pedometer steps per day were calculated based on the data recorded.



Current medications

The information on current medications was assessed by the questionnaire via the questions: “Are you currently taking any medication prescribed by a doctor?” and “Are you currently using any of the following hormonal medications?” Four response options

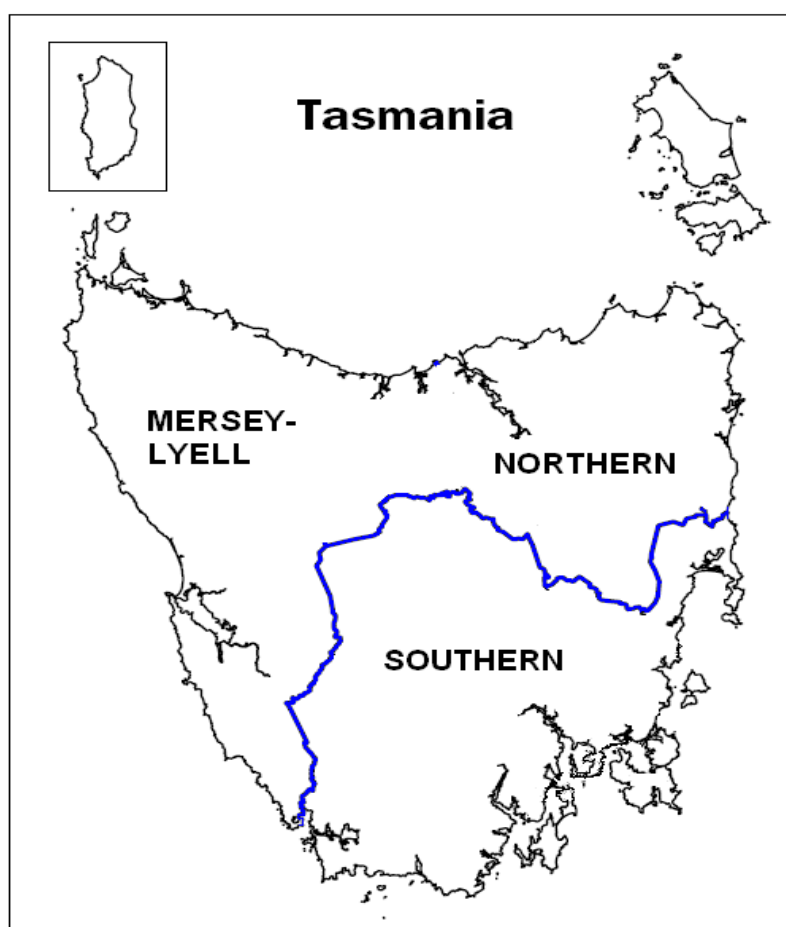
were provided: 1) I do not use any hormone medications, 2) Hormone replacement therapy, 3) Testosterone treatment (e.g. Androderm), 4) Anabolic steroids. If none of the options was applicable, participants were then asked to specify. Women who currently used hormonal medications including hormonal agents for PCOS or for in vitro fertilization (IVF) treatment were identified by checking the information they provided.

3.2 The Tasmanian Older Adult Cohort Study

3.2.1 *Study population and design*

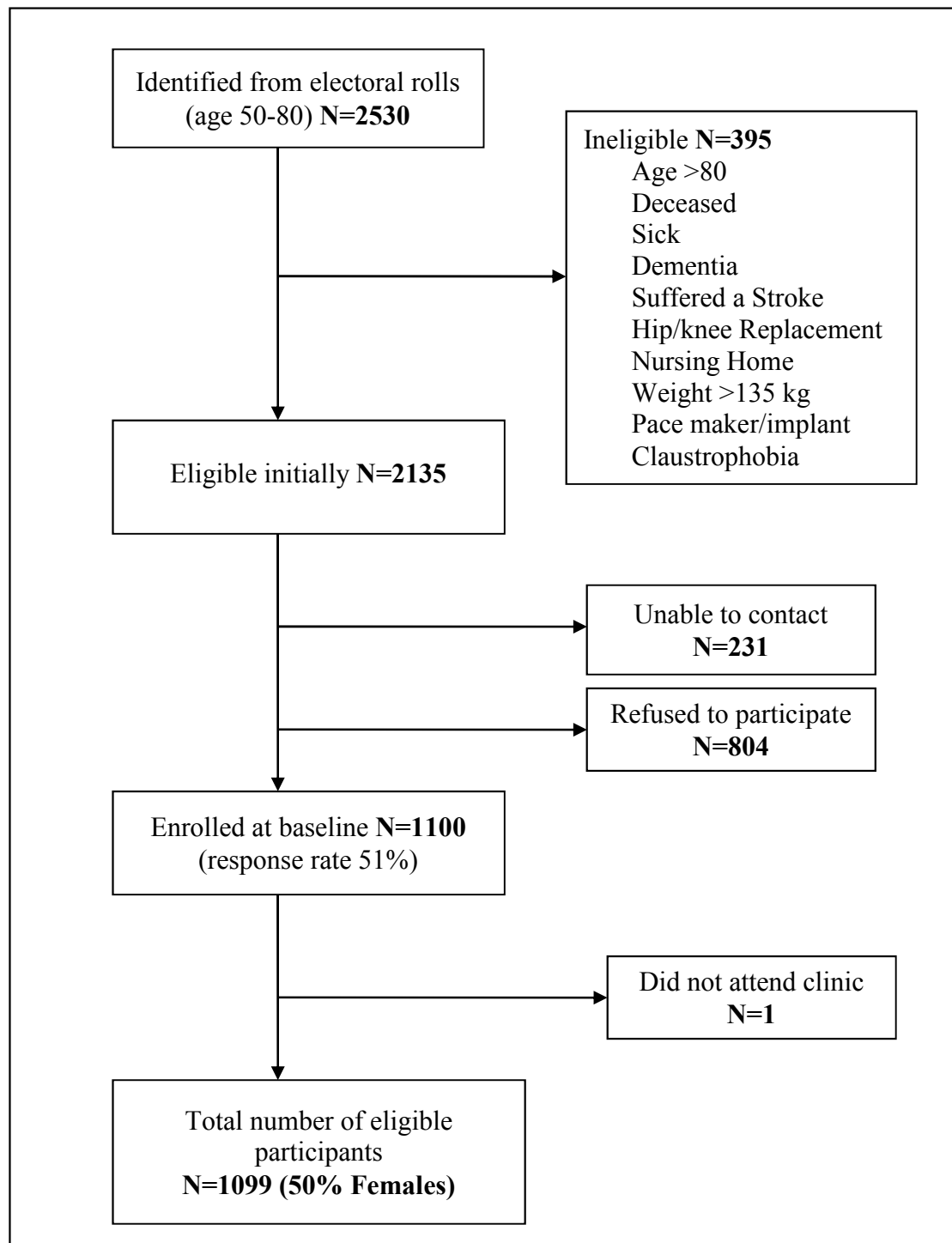
The Tasmanian Older Adult Cohort (TASOAC) study is an ongoing prospective study first commenced in 2002. Men and women between the ages of 50 and 80 years were selected randomly using computer-generated random numbers from the roll of electors in southern Tasmania (population 229,000, Figure 3.5). Electoral rolls represent the most complete population information in Australia as voting in federal and a state election is compulsory for persons aged 18 or over. An equal distribution of urban and rural areas and of men and women was selected.

Figure 3.5. Tasmanian map



The total number of subjects selected from the electoral roll was 2530. Of them 395 were initially ineligible due to being older than 80 years, deceased, unable to participate due to poor health, weighing more than 135 kg, being institutionalized, or having a contraindication for magnetic resonance imaging (MRI) such as a pace maker or implant, as participants were required to have a MRI examination. Among 2135 eligible participants, 231 of them were not contactable and 804 refused to participate. Given the total number of eligible participants for this study was 1099 (50% women), the overall response rate was 51 % (Figure 3.6). Baseline measures were conducted from April 2002 to September 2004, with further follow-up after two years and five years.

The primary aims of the TASOAC study are to determine the environmental, genetic and biochemical factors associated with the development and progression of osteoarthritis and osteoporosis. All participants provided written informed consent and the study was approved by the Southern Tasmania Health and Medical Human Research Ethics Committee (Ethics Approval Number: H0006488).

Figure 3.6. Flow chart of participants' recruitment in the TASOAC study

3.2.2 *Measurement of outcomes*

Bone mineral density (BMD)

Bone mineral density was measured by dual-energy x-ray absorptiometry (DXA) at the spine, hip and total body. DXA is known as the golden standard method for measuring BMD. The instrument used was a Hologic Delphi densitometer on array setting (Hologic, Inc., Waltham, MA). The software program was not altered during the study time frame. Bone mass was examined as bone mineral content (BMC) and areal BMD (g/cm^2), which is calculated by dividing the bone mineral content by the area measured. Precision estimates in vivo are 2–3% in this study [62].

Vertebral fracture and number wedge of fractures

Vertebral fracture was measured by DXA and assessed (SF) by using a computerized image analysis system (Hologic APEX software V2.2). One assessor assessed the scans blind to the clinical and BMD data. Using the lateral views of the DXA scans from T4 to L4, markers were placed on each of the four corners and in the centre of the superior and inferior surfaces of each vertebra in order to determine the dimensions. Data were stored as co-ordinates and exported to a database (Microsoft Access 2003 (Microsoft Corporation)), then converted to length (mm) using Pythagoras theorem. We used the ratio of the anterior to posterior lengths (heights) to determine whether a vertebral fracture was present or not, defined as a ratio of <0.8 , representing $\geq 20\%$ reduction in height of the anterior portion of a vertebral body relative to the posterior height of that body. Persons with $\geq 20\%$ reduction in vertebral height are able to access anti-osteoporotic pharmaceuticals through the Australian Pharmaceutical Benefits Scheme [63]. We excluded participants from the morphometry analyses if <10 vertebrae were visualized on the scan ($n=3$). The total number of wedge fractures (T4–L4) and presence or absence of fractures in any vertebrae (T4–L4) were recorded and used in the analysis.

Cartilage volume

Participants underwent MRI scans of the right knees to determine cartilage volume. Knees were imaged in the sagittal plane with a 1.5T whole-body magnetic resonance unit (Picker, Cleveland, OH) using a commercial transmit-receive extremity coil. The following image sequence was used: a T1-weighted fat-suppressed 3-dimensional gradient-recall acquisition in the steady state, flip angle 55° , repetition time 58 msec, echo time 12 msec, field of view 16cm, 60 partitions; 512×512 -pixel matrix, acquisition time 11 minutes 56 seconds, and one acquisition. Sagittal images were obtained at a partition thickness of 1.5 mm and an in-plane resolution of 0.31×0.31 mm (512×512 pixels).

Knee cartilage volume was determined by means of image processing on an independent computer work station using Osiris software (University of Geneva, Geneva, Switzerland). The volumes of individual cartilage plates (medial tibial, lateral tibial, medial femoral, lateral femoral and patella) were isolated from the total volume by manually drawing disarticulation contours around the cartilage boundaries on a section-by-section basis. These data were then resampled by means of bilinear and cubic interpolation (area of 312×312 μm and thickness of 1.5 mm, sing continuous sections) for the final 3-dimensional rendering. The volume of the particular cartilage plate was then determined by summing all the pertinent voxels within the resultant binary volume. There was high intra-observer and inter-observer reproducibility with this method. Inter observer reproducibility was assessed in 30 knees by the same reader twice, approximately one month apart. The coefficient of variation for the cartilage volume measures was 2.1% for the medial tibial and 2.6% for the lateral tibial [64].

Knee femoral cartilage volume was determined using CartiscopeTM (ArthroVision Inc., Montreal, Canada) running on a Windows NT/9x workstation, as previously described [65-68]. Cartilage volume was evaluated directly from a standardized view of 3D cartilage geometry as the sum of elementary volumes. The CV was about 2% [67].

Cartilage defects

Cartilage defects were assessed by one observer (CD) as mean of image processing on an independent workstation using Osiris. Cartilage defects on a 0-4 scale were graded at the medial tibial, medial femoral, lateral tibial, lateral femoral and patella sites as follows [69, 70]: grade 0=normal cartilage; grade 1=focal blistering and intracartilaginous low-signal intensity area with an intact surface and bottom; grade 2=irregularity on the surface or bottom and loss of thickness of <50%; grade 3=deep ulceration with loss of thickness >50%; grade 4=full-thickness chondral wear with exposure of subchondral bone. A cartilage defect had to be present in at least two consecutive slices. The cartilage was considered to be normal if the band of intermediate signal intensity had a uniform thickness. Presence of cartilage defects were defined as a score of ≥ 2 at any site measured at the knee (within that compartment). Intra-observer reliability expressed as intra-class correlation coefficient (ICC) was 0.89-0.94, and inter-observer reliability was 0.85-0.93 [71].

Tibial bone area was determined at the medial and lateral compartments as previously described [23]. To transform the image from the sagittal to the axial plane, the Analyses Software package developed by the Mayo Clinic (Rochester, MN) was employed. Medial and lateral tibial plateau bone area was determined by creating an isotropic volume from the three input images closet to the knee joint line after reformatting in the axial plane (approximately 5 mm from the joint line). The bone area of the medial and lateral plateau was then directly measured from the reformatted axial images. The CVs obtained for these measurements were 2.2-2.6% [23].

Joint space narrowing and osteophytes

Joint space narrowing (JSN) and osteophytes were assessed by X-ray by using a standing anteroposterior view of the right and left knee in a semiflexed position. This protocol has been described previously [23]. According to the standard of the Osteoarthritis Research Society International atlas [72], JSN and osteophytes were both assessed on a scale of 0-3 which is from no disease (normal) to severe disease in the compartment of medial tibiofemoral JSN, lateral tibiofemoral JSN, medial tibial

osteophytes, medial femoral osteophytes, lateral tibial osteophytes, and lateral femoral osteophytes. Intra-observer repeatability was assessed and the intra-class correlation (ICC) was 0.65-0.85 [23]. The presence of osteophytes was defined as any score ≥ 1 whereas JSN was defined as any score ≥ 2 in the tibiofemoral compartments of either knee.

3.2.3 *Measurement of study factors*

Parity

Parity was assessed by self-administered questionnaire (Appendix 8) in this study. To obtain numbers of pregnancies and births, the questions were used: “Have you ever been pregnant?”, “How many times have you been pregnant?”, “How many times have you had a miscarriage/termination?” and “How many times have given birth to a child (live or stillborn)?” Parous was defined if the participant had had at least birth. Parity was determined based on the number of births and then categorized as four groups (1 = nulliparous, 2 = one or two children, 3 = three or four children, and 4 = 5 or more) to ensure similar proportions in each group.

Ever use of oral contraceptive

Ever use of OC status was determined by the same questionnaire. The question asked “have you ever used the oral contraceptive pill?” If the answer was yes, then the following question was asked: “How many years in total have you ever taken the oral contraceptive pill?” There were six optional responses available which ranged from never, less than 1 year, 1-4 years, 5-10 years, 11-20 years and more than 20 years. Based on the information, duration of OC use was categorized as never use, less than five years use, five to ten years use and more than ten years use to obtain similar proportions for each group and to simplify analyses.

Use of hormone replacement therapy

Use of hormone replacement therapy status was also recorded by the questionnaire. Women were asked: “Are you currently on hormone replacement therapy (HRT)?” and “For how many years in TOTAL have you ever used hormone replacement therapy?” Five

optional answers were available that included never use, less than 1 year, 1-4 years, 5-10 years, and more than 10 years. Use of HRT was assessed as either current or ever use against never use based on the information provided. Duration of use of HRT was again grouped as never use, less than five years use, five to ten years use and more than 10 years use.

3.2.4 *Measurement of covariates*

Menopause

Information on menopause status was obtained by asking the questions: “Have your periods NOW stopped for more than 12 months?”, “When did your periods stop?”, “Have you had a hysterectomy?” and “Have you ever had an operation to remove both ovaries?” Menopause was defined based on these criteria: 1) women with intact uterus and ovaries and menstrual cycles stopped for more than 12 months; 2) women who had uterus and both ovaries removed; 3) women who had a hysterectomy but at least one ovary retained and aged over 55 years.

Smoking

Smoking history was determined by the questionnaire using questions that included the following: “Have you ever been a regular smoker?” and “Are you currently a regular smoker?” A regular smoker was described to participants as someone who smoked at least seven cigarettes, cigars or pipes every week for three months or more. Smoking was re-assessed as ever smoker if they were current smokers or former smokers, or never smoker if they had never been regular smokers.

Alcohol consumption

The information on alcohol consumption was collected from participants by the Food Frequency Questionnaire (FFQ, Appendix 9) developed by the Cancer Council Victoria [73]. This questionnaire considered drinks including beer, wine and spirits that participants consumed over the last 12 months. Participants were asked: “Over the last 12 months, on days when you were drinking, how many glasses of beer, wine and/or spirits altogether did you usually drink?” To answer this question, participants were asked to

calculate the amounts drunk by glasses using the examples given in the questionnaire and count each nip (30ml) as one glass, and were also informed: 1 can or stubby of beer = 2 glasses, 1 large bottle beer (750 ml) = 4 glasses, 1 bottle wine (750ml) = 6 glasses and 1 bottle of port or sherry (750ml) = 12 glasses. Alcohol consumption was recorded as total number of glasses per day.

Anthropometry

Height was measured to the nearest 0.1 cm using a Leicester stadiometer (Invicta, Leicester, UK), with shoes, socks and headgear removed. Weight was measured to the nearest 0.1kg using a single pair of electronic scales (Delta Model 707; Seca, Hamburg, Germany), with shoes, socks, and bulky clothing removed. The electronic scales were calibrated using a known weight at the beginning of each clinic. Body mass index (BMI) was calculated as weight (kg) divided by height (cm) in meters squared (kg/cm^2).

Physical activity

Physical activity was assessed as steps/day determined by pedometer (Omron HJ-003 & HJ-102, Omron Healthcare, Kyoto, Japan). Pedometers were calibrated at the clinic with the participants present, using a 100-pace walking test. A pedometer diary and written instructions regarding pedometer wear and how to keep a pedometer diary were issued to participants. Participants were requested to wear pedometers for seven consecutive days except when bathing, sleeping or water based activities, and follow their normal daily routine. Based on recorded diaries, an average of the seven days was used to give each participant a mean steps/day value. Pedometer readings were excluded if they were determined to be caused artificially (e.g. report of work done on heavy machinery), or if the pedometer had been worn for less than five days.



WOMAIc-pain score

Knee pain was assessed by self-administered questionnaire (Appendix 10) using Western Ontario and McMaster University index (WOMAC) [74] . Five categories of pain

(walking on flat surface, going up/down stairs, pain at night, sitting/lying, and standing upright) were assessed separately with a 10-point scale from 0 (no pain) to 9 (most severe pain) for each category. A total pain score was sum of these scales with range from 0 to 45.

3.3 The CDAH Knee Cartilage Study

3.3.1 *Study population and design*

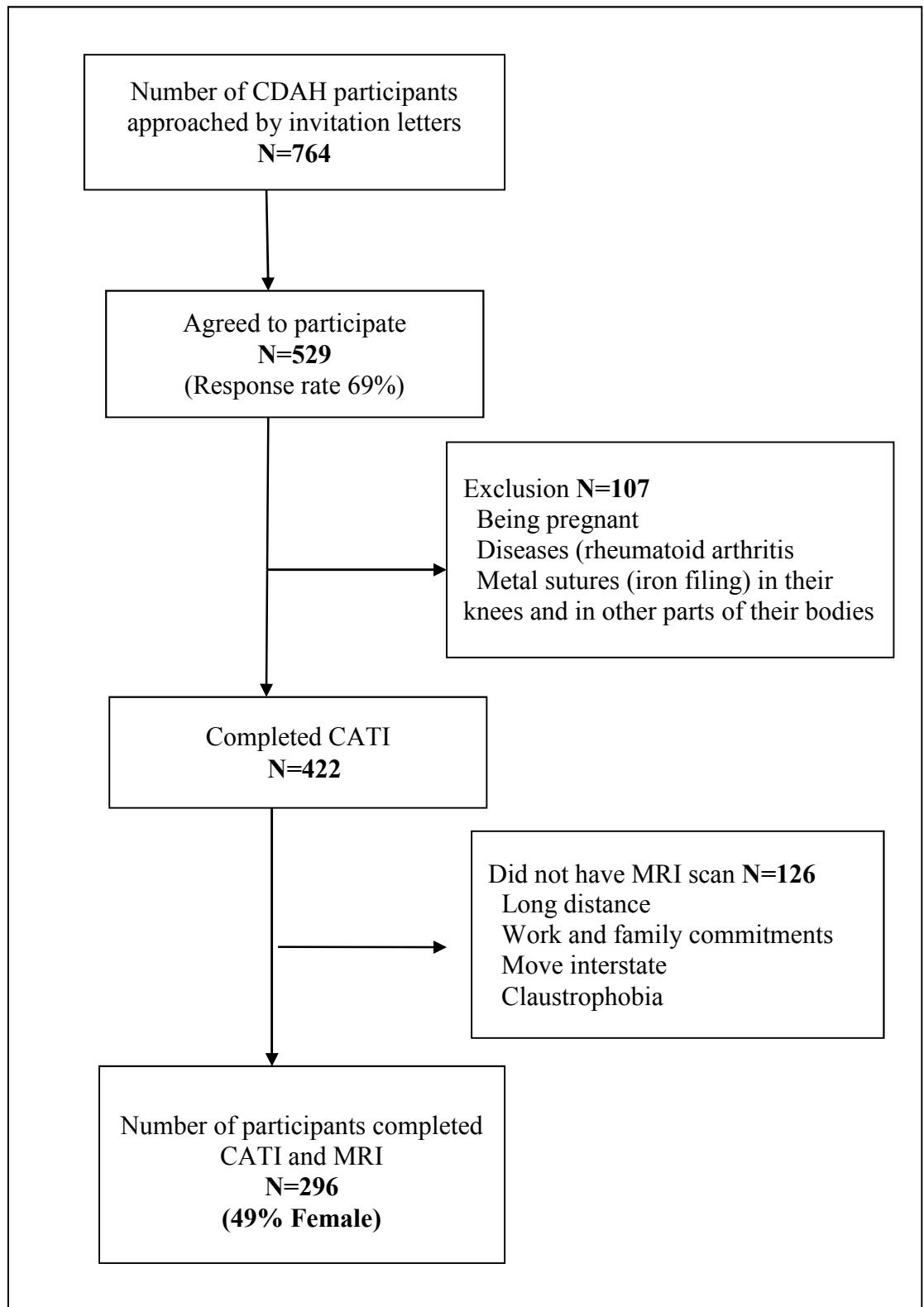
The CDAH Knee Cartilage Study is a follow-up of a sub-sample of participants who completed the Childhood Determinants of Adult Health (CDAH) study in 2004 to 2006. Following earlier findings from the CDAH study that childhood physical fitness and fatness were associated with adult BMD [55], this study was designed to investigate the effects on adult knee cartilage. The CDAH Knee Cartilage study occurred from April 2008 to December 2010 by mailing out invitation letters to the CDAH participants (n=746) who lived in metropolitan Melbourne or Sydney, Australia. Women who agreed to participate were asked to complete short computer assisted telephone interviews (CATIs) (Appendix 11) and eligible participants were requested to have an MRI scan at either Epworth Hospital in Melbourne or North Shore Private Hospital in Sydney.

Exclusion criteria for this study included being pregnant, having had diseases which may affect knee cartilage, such as rheumatoid arthritis, and having contraindication for MRI such as metal sutures (iron filing) in their knees or in other parts of their bodies. Women who had no MRI scan were further excluded from the analyses. Reasons for refusing MRI results included the long distance needed to travel for MRI, work and family commitments, moving interstate, and claustrophobia.

Among 764 approached CDAH participants, 529 (69%) agreed to participate in the follow-up study and 422 completed CATIs. Of these, 296 participants had an MRI scan (49% women) and were eligible for this study (Figure 3.7).

All participants provided written informed consent and this study was approved by the Southern Tasmania Health and Medical Human Research Ethics Committee (Ethics Approval Number: H0009828).

Figure 3.7. Flow chart of participants' recruitment in the CDAH Knee Cartilage study



3.3.2 *Measurement of outcomes*

Cartilage volume

Each participant had an MRI scan of the dominant knee defined as the lower limb from which the subject stepped off when initiating gait. Two hospitals were involved in the scans but the same type of machine (General Electric Medical Systems, Milwaukee, WI, USA) was employed. Knees were imaged in the sagittal plane on a whole body magnetic resonance unit with use of a commercial transmit-receive extremity coil. The following image sequence was used: a T1-weighted fat saturation 3D gradient recall acquisition in the steady state; flip angle 55 degrees; repetition time 58 msec; echo time 12 msec; field of view 16 cm; 60 partitions; 512 x 512 matrix; acquisition time 11 min 56 sec; one acquisition. Sagittal images were obtained at a partition thickness of 1.5 mm and an in-plane resolution of 0.31 x 0.31 (512 x 512 pixels).

Individual plates of cartilage volume (medial tibial, lateral tibial and patella) were isolated from the total volume by manually drawing disarticulation contours around the cartilage boundaries on a section by section basis. These data are then re-sampled by means of bilinear and cubic interpolation (area of 312 μm and thickness of 1.5 mm, continuous sections) for the final 3D rendering. The coefficients of variation (CVs) for cartilage volume measures were 2.1-2.6% [23].

Seven subjects had MRI scans for cartilage volume and bone area performed at both hospitals and data from these scans were used to correct all readings for bias. Bland-Altman plots were used to examine differences between hospitals and determine the nature of any observed bias. Uniform biases were identified for lateral and medial cartilage volume, and remedied by applying a difference correction based on the mean difference. Value dependent bias (patella cartilage volume) was remedied by applying corrections using the slope and intercept from a fitted linear regression model. Patellar-tibial cartilage volume was calculated by summing cartilage volumes at three sites (patellar, medial tibial and lateral tibial) together. One reader assessed cartilage measures and was blinded to parity status of the women.

Cartilage defects

Cartilage defects were graded at the medial and lateral tibial, medial and lateral femoral and patella sites as grade 0 to 4 (from normal to severe) as described in the TASOAC study. A cartilage defect had to be present in at least 2 consecutive slices and was defined as a score of ≥ 2 at any site measured at the knee (within that compartment). Intra-observer reliability expressed as intraclass correlation coefficient (ICC) was 0.89-0.94, and inter-observer reliability was 0.85-0.93 [71].

3.3.3 *Measurement of study factors*

Parity

Reproductive factors were not assessed in CDAH Knee Cartilage Study as this was not the primary aim of the study. However, information on parity was available from a second follow-up survey of the CDAH cohort (CDAH-2) which collected data by postal or online questionnaire, or telephone interview during 2009-2011 (Appendix 12). The aims of CDAH-2 were to investigate associations between life-stage transitions, depression and cardio-metabolic disease risk in young adults.

The numbers of biological children and date of birth were recorded following the questions: “How many biological children have you had?” and “When were they born?” Births were counted only if these occurred before knee MRI was performed. Parity was then defined based on the numbers of births (twins only counted as singular). Women were also categorized as nulliparous or parous if they had at least one live birth.

Other reproductive factors were also assessed by the questionnaire including age at menarche and current use of OC status. OC use was categorized as no-current hormonal contraceptive use, current OC use and current progesterone only contraceptive use as described in the CDAH study.

3.3.4 *Measurement of covariates*

Body mass index

Weight was measured to the nearest 0.1kg using a single pair of electronic scales (Delta Model 707; Seca, Hamburg, Germany), with shoes, socks, and bulky clothing removed. The electronic scales were calibrated by using a known weight before each clinic commenced. Height was measured at CDAH study clinics when participants were aged 26-36 by using Leicester Height Measure (Invicta, Leicester, UK) with shoes removed and this was assumed to be unchanged at the time of the CDAH Knee Cartilage Study. Body mass index (BMI) was calculated as weight (kg) divided by height (cm) in meters squared (kg/cm^2).

Physical activity

Physical activity was assessed by the CATI using the short international physical activity questionnaire (IPAQ) [60]. The IPAQ-short considered three levels of physical activities including vigorous, moderate and walk that participants performed for at least 10 minutes in the duration for the last seven days, and recorded frequency, duration and intensity of physical activity that they participated. Total physical activity was calculated as a score by the equation of Metabolic Equivalent Task (MET, minutes/week) based on the time spent over the 7 days and types of the physical activities which including vigorous, moderate and walk assessed from the IPAQ short questionnaire [60].

Smoking history

Smoking was assessed by the CATI questionnaire. As this study is a follow-up of CDAH participants, the CATI questionnaire focussed on the changes in smoking behaviours thus the questions used were: “Have you changed your smoking status since your last interview for this study?” and “if you started smoking, how often do you smoke cigarettes, cigars, pipes or any other tobacco products?” Smoking status was categorized as never or ever smoker by matching the information provided from the CATI and the CDAH study.

Knee injury

Knee injury was assessed by the CATI by using the question: “Have you had a knee injury requiring non-weight bearing treatment for more than 24 hours or surgery during childhood?” and “Have you had a knee injury requiring non-weight bearing treatment more than 24 hours or surgery in your adult life?” Knee injury was categorized as a dichotomized variable and taken into account if injury had occurred during childhood or in adult life.

WOMAC-pain score

Knee pain was assessed by CATI using Western Ontario and McMaster University index (WOMAC) [74] . Five categories of pain (walking on flat surface, going up/down stairs, pain at night, sitting/lying, and standing upright) were assessed separately with a 10-point scale from 0 (no pain) to 9 (most severe pain) for each category. A total pain score was sum of these scales with range from 0 to 45.

Highest education achieved and Employment status

The highest education level achieved was assessed by the general questionnaire (CDAH-2) via using the question: “What is the highest level of education you have completed?” The levels were then collapsed to school only (from primary school to year 12 or equivalent), diploma/vocational training (including trade, apprenticeship, certificate and diploma) or university education (including university degree and higher university degree. Current employment status was obtained by the CATI though the question: “Which of the following describes your current employment status?” Employment status was categorized as full-time employment, part-time employment and not in workplace which including home duty, student and not working).

Bone area

The area of medial and lateral tibial plateau bone was measured manually on the three reformatted images closest to tibial cartilage in the axial plane which was transformed from the sagittal images as described in the TASOAC study. An average of

these three areas will be used as an estimate of the tibial plateau bone area. The CVs for these measures were 2.2-2.6% [23].

3.4 Data analysis

Details of the statistical analysis are presented in the relevant chapters. All statistical analyses were performed on Intercooled Stata versions 9.2 or 10.1 for Windows (Statacorp, Texas, USA).

3.5 Summary of study samples, study factors and outcomes by chapters

Table 3.1 summarizes the study samples used and the key outcomes, study factors and covariates in each Chapter of this thesis.

Table 3.1. Summary of outcomes, study factors and covariates by chapters

Chapter	Study	Outcomes	Study factors	Covariates
4	CDAH	Bone mass (BUA, SOS, QUI)	Use of OC/progestin- only contraceptive	Age, BMI, leisure time PA, parity, PCO/PCOS, menstrual regularity
5	TASOAC	Spine, total body and hip BMD, vertebral deformity	Use of OC and duration	Age, BMI, pedometer steps, alcohol, consumption, menopause, use of HRT
6	CDAH	Bone mass (BUA, SOS, QUI)	Menstrual regularity and hormonal factors	Age, BMI, smoking, PCO/PCOS
7	TASOAC	knee cartilage volume and the defects, JSN, osteophytes	Parity, use of OC and HRT	Age, BMI, smoking, WOMAC pain score, knee surgery
8	CDAH Knee Cartilage	knee cartilage volume and the defects	Parity	Age, BMI, smoking, total PA, knee injury, bone area

CDAH= Childhood Determinants Adult Health study. TASOAC=Tasmanian Older Adult Cohort study. BUA=broadband ultrasound attenuation. SOS=speed of sound. QUI=quantitative ultrasound index. OC=oral contraceptive. BMI=body mass index. PA=physical activity. PCO= polycystic ovaries. PCOS=polycystic ovary syndrome. BMD=bone mineral density. HRT=hormone replacement therapy. JSN=joint space narrowing. WOMAC=Western Ontario and McMaster University index.

Chapter 4

Oral Contraceptive Use and Bone Mass in Women Aged 26-36 Years

Published in the

Osteoporosis International. 2011, 22 (1):351-355

4.1 Introduction

The association between hormonal contraceptive use, bone mineral density (BMD) and risk of osteoporotic fracture remains controversial [75]. A systematic review of studies of combined hormonal contraceptive use and BMD found generally lower BMD in adolescents and young women (≤ 22 years) currently using combined oral contraceptives (OC) compared with nonusers, but generally no difference in BMD in premenopausal women in a broader age range. Current combined OC use in peri-menopausal and postmenopausal women (eg in randomised trials) was positively associated with BMD, but was not different between former OC users and never users in this older group [75]. More recently, several studies have examined the associations in young women are also presented inconclusive results [18, 76-78], with negative associations in samples recruited from a university or from fit, heavily-exercising female military cadets [18, 76], and a beneficial effect in female athletes over 2 years follow-up [77]. Progesterone only contraceptives, such as depot medroxyprogesterone acetate (DMPA), have been associated with lower BMD in young women [79] but studies of other types of progestogen-only contraceptives and BMD are limited [80]. The associations between OC use and risk of fractures are mixed though DMPA users were more likely to experience stress fractures than nonusers [80].

The purpose of this study was to describe the associations between hormonal contraceptive use and bone mass measured by quantitative ultrasound (QUS) in a large population-based sample of premenopausal women aged 26-36 years.

4.2 Materials and methods

4.2.1 Subjects

This study used data from the Childhood Determinants of Adult Health (CDAH) study, a 20-year follow-up of children who participated in 1985 Australian Schools Health and Fitness Survey (ASHFS) at age 7-15 years. Details of the 1985 sampling strategy have been described elsewhere [43]. Briefly, a two-stage probability sampling technique was employed to first select schools with a probability proportional to the number of 10 year-olds in primary schools and 14 year-olds in secondary schools (n=109, 90.1% response rate). The second stage of sampling involved selecting children within age and sex strata within each school using simple random sampling (n=8498, 67.5% response rate). During follow-up (2004-2006) a total of 2410 subjects aged 26-36 years (48% male, 52% female), completed questionnaires and attended one of 34 study clinics in major cities and regional centres around Australia for extensive physical measurements including anthropometric measures, quantitative ultrasound and blood biochemistry. The CDAH study aims to investigate childhood factors associated with chronic disease risk; primarily cardiovascular disease and diabetes, but also including musculoskeletal disorders [55].

Eligible subjects for this study were women who had completed study questionnaires, attended clinics, and were not currently pregnant or breast feeding. Of the 1260 women who attended clinics, 82 were currently pregnant, 33 did not provide information on contraceptives use, 454 had missing QUS measurements due to the machine being out of service, and 4 were taking hormonal medications that were thought to be associated with bone metabolism, leaving a total of 687 participants. All participants provided written informed consent and the study was approved by the Southern Tasmania Health and Medical Human Research Ethics Committee.

4.2.2 Assessment of contraceptive use and other study characteristics

Women's use of hormonal contraceptives was obtained by self-administered questionnaire. The question was: "are you currently using any of the following hormonal contraceptives, even if you are using them for reasons other than contraception?"

Contraceptive status was then categorized as follows: oral contraceptive users (OC-user) if women were currently using a combined oral contraceptive; progestogen-only contraceptive users (progestogen-user) if they were currently using any progestogen-only contraceptives (including oral, injectable and implanted types and progestin releasing intrauterine devices); and non-hormonal contraceptive users if women were not currently using any hormonal contraceptives. Women were defined as having a history of menstrual irregularity if they described their period as irregular or very irregular during the most recent time when they were not using hormonal contraceptives and were not pregnant or breastfeeding.

Demographic and lifestyle information was also obtained by questionnaire. Smoking status was categorized as either non-smoker or current smoker. Women were classified as either nulliparous or parous if they had had at least one live birth. Daily serves of dairy items, alcohol consumption and calcium supplement use were measured by food frequency questionnaire. Leisure time physical activity (LTPA) was assessed as we have described previously [81] using the International Physical Activity Questionnaire and as mean steps per day by Yamax pedometer worn for seven days. The questionnaire asked whether participants had ever been told by a doctor that they had polycystic ovaries (PCO) or polycystic ovary syndrome (PCOS).

4.2.3 *Measurement of bone mass and anthropometrics*

Bone mass was measured by quantitative ultrasound (QUS) at the heel by a single Sahara Clinical Bone Sonometer (Hologic Inc., MA, USA). The ultrasound system consists of two sound transducers mounted coaxially on a motorized calliper where one transducer acts as an emitter and the other as a receiver. This makes direct contact with the heel through elastomer pads and an ultrasonic coupling gel. The lower part of the dominant leg was placed immobilized during measurement and the proper leg angle set by a positioning aid. Broadband ultrasound attenuation (BUA, dB/MHz) and speed of sound (SOS, m/s) were measured at a fixed region in the mid-calcaneus. The quantitative ultrasound index (QUI) was derived from SOS and BUA using the equation: $QUI = 0.41 \times (BUA + SOS) - 571$. Quality assurance was performed daily by calibrating the device on a dedicated phantom

supplied by the manufacturer. The coefficient of variation (CV) for QUS measures was 1%. As ambient temperature affects measurements, this was collected for each clinic and adjusted for in all analyses.

Height and weight were measured to the nearest 0.1cm and 0.1 kg, respectively, and body mass index (BMI) was calculated as the ratio of weight (kg) to height (m) squared (kg/m^2). Waist to hip ratio (WHR) was calculated by dividing waist by hip circumference measured to the nearest 0.1cm.

4.2.4 *Statistical Analysis*

The mean differences of continuous characteristics across contraceptive use status (non-user, progestogen only user and combined OC-user) were assessed by regression models with logarithmically transformed values for skewed data; the Chi square test or Fisher's exact test was used for dichotomous variables. The ambient temperature was significantly associated with all QUS measures ($p < 0.05$). To adjust for this, ambient temperature was regressed on each of the QUS parameters, and the residual values calculated for each QUS measurement used in the analysis [55]. Multivariable linear regression analysis was used to explore the association between contraceptive use and QUS measures. Outcome measurements were logarithmically transformed to fit regression models and standardized to allow direct comparisons between BUA, SOS and QUI. Age, BMI, leisure time physical activity and parity were considered as potential confounders and adjusted for in all multivariable models as they were significantly associated with both contraceptive status and QUS measurements. All statistical analyses were performed on intercooled Stata 9.2 for windows (Statacorp, Texas, USA).

4.3 Results

In this study sample (aged 26-36 years), 62% of participants were non-users of hormonal contraceptives, 32% were current combined OC-users and only 6% were progestogen-only contraceptive users. Women with missing data on QUS were slightly younger (30.5 years vs 31.2 years) than those included in the analysis but had no significant differences in BMI, daily serves of dairy items and physical activity (PA) (all $P>0.05$), and similar proportions of contraceptive user, smoking, parity and history of menstrual irregularity (all p values >0.05).

The characteristics of study participants across contraceptive groups are shown in Table 4.1. OC-users were younger, had lower BMI, were more likely to have a history of menstrual irregularity, were more physically activity in leisure time but similar with respect to pedometer steps, and more likely to be unmarried and nulliparous than non-users. Compared with non-users, OC-users had significantly higher QUS including BUA, SOS and QUI. There were no significant differences between combined OC-users and non-users in alcohol consumption, daily serves of dairy items, smoking status and calcium supplement use. Women using progestogen-only contraceptives ($n=43$) were more likely to have a higher BMI and waist circumference than non-users but comparisons of other characteristics were not meaningful in this small sample.

Adjusted associations between oral contraceptive use and bone mass are shown in Table 4.2. Compared with non-users, women using oral contraceptives had significantly higher values of BUA, SOS and QUI. The relative effect of OC use was similar for BUA, SOS and QUI (standardized coefficient was 0.21, 0.23 and 0.23 respectively). These associations remained after adjustment for age, BMI, LTPA and parity, with an increase of 0.18 in the standardized log-transformed BUA for OC users. This corresponds to a 4% increase in the mean BUA (dB/MHz). Further adjustment for a history of PCO, PCOS or menstrual irregularity did not alter these results (data not shown). Progestogen-only contraceptive users also had higher BUA than non-users, but the differences were not statistically significant in this small group.

Table 4.1. Characteristics of participants by contraceptive use

	Contraceptive use			<i>P</i> value
	Non -user (N=425)	Progestogen-user (N=43)	Combined OC- user (N=219)	
Age ^a(years)	31.5 (2.5)	31.3 (2.7)	30.5 (2.8)	0.001
Body composition ^a				
Weight (kg)*	68.7 (15.6)	75.0 (13.5)	66.6 (11.2)	0.06
Height (cm)	165.8 (6.0)	166.7 (6.2)	165.5(6.5)	0.48
BMI (kg/m ²)*	24.9 (5.2)	27.1 (5.4)	24.3 (4.0)	0.11
Waist circumference (cm)*	79.0 (12.0)	83.7 (11.3)	76.7 (8.6)	0.01
Current smoker (%)	13.9	11.9	13.5	0.49
Married or living as married (%)	61.6	69.8	42.3	<0.001
Parous (%)	52.8	67.4	34.7	<0.001
History of menstrual irregularity (%)	11.4	18.6	21.8	<0.01
Ever been told PCO or PCOS (%)	4.9	9.3	5.9	0.58
Physical activity (PA)^b				
Leisure time PA	90 (0-210)	100 (20-240)	150 (60-280)	0.03
(min/week)	8392 (6730-10534)	8037 (6803-9414)	8895 (7187-10661)	0.14
Pedometer steps (per day)				
QUS measurement ^b				
BUA (dB/MHz)	74.0 (65.9-84.6)	78.2 (69.8-84.2)	77.7 (68.6-87.5)	0.01
SOS (m/s)	1565(1544-1588)	1567 (1550-1576)	1571 (1548-1594)	0.01
QUI (%)	100.2(89.2-114.8)	102.6(93.9-111.8)	105.9 (93.3-118.9)	0.01

P values for differences between OC-user and non-user are derived from regression models for continuous variables, Chi² or Fisher's exact test for dichotomous variables. Non-users included women who were not on any hormonal contraceptives. Progestogen-users included women who were using progestogen-only contraceptives (such as injectable and

implanted progestogen). Combined OC-users were women who use combined oral contraceptives. BMI=body mass index. PCO= polycystic ovaries. PCOS=polycystic ovary syndrome. QUS=quantitative ultrasound. BUA=broadband ultrasound attenuation. SOS=speed of sound. QUI=quantitative ultrasound index. * $P < 0.05$ between progestogen-user against non-user. ^a mean and standard deviation (SD), ^b median and inter-quartile range.

Table 4.2. Standardized coefficients from linear regression models of contraceptive use and bone mass measured by quantitative ultrasound (QUS)

Contraceptive use	Model 1			Model 2		
	BUA	SOS	QUI	BUA	SOS	QUI
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Non-user	Ref	Ref	Ref	Ref	Ref	Ref
Progestogen-user	0.19 (-0.12, +0.50)	-0.03 (-0.34, +0.28)	0.07 (-0.24, +0.38)	0.16 (-0.16, +0.47)	0.03 (-0.29, +0.35)	0.09 (-0.22, +0.42)
Combined OC-user	0.21 (+0.04,+0.37)	0.23 (+0.07, +0.39)	0.23 (+0.07, +0.39)	0.18 (+0.01, +0.35)	0.16 (-0.01, +0.34)	0.18 (+0.01,+0.35)

Model 1 QUS measures adjusted for ambient temperature only. Model 2 further adjusted for age, BMI, leisure time physical activity and parity. Non-users included women who were not using any hormonal contraceptives. Progestogen-users included women who were using progestogen-only contraceptives (such as injectable and implanted progestogen). Combined OC-users were women who use combined oral contraceptives. QUS measures were logarithmically transformed before being standardized. Ref=reference group. BUA=broadband ultrasound attenuation. SOS=speed of sound. QUI=quantitative ultrasound index. β =standardized beta coefficients. Bold-face figures denote statistically significant difference (at $p<0.05$).

4.4 Discussion

Combined oral contraceptive users had significantly higher bone mass measured by quantitative ultrasound including BUA, SOS and QUI in this population based sample of women aged 26-36 years. These associations were independent of age, BMI, physical activity and parity and were unaffected by history of menstrual irregularity, PCO or PCOS. Women using progestogen-only contraceptives seemed to have higher BUA than non-users but the small number of women in this group limited the inferences that could be drawn.

Previous cross-sectional studies examining the association between OC use and BMD in samples of young women (≤ 25 years) have had conflicting results with findings of no association in relatively small samples [19, 82] or a negative associations in samples recruited from a university setting or from fit, heavily-exercising female military cadets [18, 76]. Our study findings contrast with these studies but are consistent with other reports on the associations between combined oral contraceptive use and bone mineral density. Cross-sectional findings from 476 young women aged 25-37 years in the CARDIA study found that cumulative estrogen exposure from OCs was positively associated with BMD in white but not black women [83]. Another large population based study of 2474 women aged 30-34 years in 7 developing countries also found that current OC users had significantly higher BMD than those who never used hormonal contraceptives [84]. They are also consistent with an Australian retrospective study in which ever use of OCs was associated with higher BMD in 511 premenopausal women [85]. The different results between studies might be explained by different characteristics of participants (such as age); differences in dose, formulation or duration of OC use; different techniques used in the measurement of BMD; or ability to adjust for confounders.

Injectable progestogen-only contraceptives, such as DMPA, may have a negative effect on BMD [86] possibly via estrogen suppression. However the associations between progestogen implants and BMD are again conflicting [87]. Studies of other types of progestogen-only contraceptives were few and restricted to lactating women [88]. Our data

suggested a weak positive association between progestogen-only contraceptive use (all types combined) and bone mass which is consistent with a recent review that use of progestogen-only contraceptives other than DMPA did not affect BMD [80]. However, our sample of progestogen-only users was too small to draw firm conclusions.

As well as the strength of this study with its relatively large population-based sample of young premenopausal women, there are also several limitations in our study. In relation to study measures, we asked only about women's current contraceptive use and were unable to determine past use or the duration of use. The associations observed could be underestimated if OC use has an ongoing effect. Most recent menstrual cycle characteristics were obtained by self-administered questionnaire which did not include specific criteria for women to assess their cycle regularity and this may have resulted in misclassification. We did not have dual-energy x-ray absorptiometry (DXA) measurement of BMD; however QUS results correlate well with BMD measured by DXA at the heel [53] and predict fracture risk similarly to DXA [54]. The relatively low percentage of eligible participants with complete data means that our results might not apply to the general population. We could not be confident about any associations between progestogen-only contraceptive use and bone mass as only small numbers of women were in this group. Other limitations include our inability to measure potential confounders such as sex hormone levels and the study's cross-sectional design which limits inferences about the causal direction of the associations.

In conclusion, combined oral contraceptive use was associated with higher bone mass measured by quantitative ultrasound in this population-based sample of premenopausal women aged 26-36 years while progestogen-only contraceptives appeared to have no deleterious effect on bone mass.

Chapter 5

The Association between Oral Contraceptive Use, Bone Mineral Density and Fractures in Women Aged 50-80 Years

Published in the

Contraception. 2011, 84 (4):357-362

**Chapter 5 has been removed due to copyright or
proprietary reasons**

Chapter 6

Menstrual Irregularity and Bone Mass in Premenopausal Women: Cross-sectional Associations with Testosterone and SHBG

Published in the

BMC Musculoskeletal Disorders. 2010, 11:288

6.1 Introduction

Osteoporosis is a major public health concern [89]. Most osteoporosis sufferers are postmenopausal women and age-related estrogen deficiency is considered a major cause of bone loss [17]. As well as estrogen, androgens may also have an effect on bone metabolism in women; excess levels of androgen are associated with higher bone mass in women with Polycystic Ovary Syndrome (PCOS) [15, 16, 120-122]. Menstrual irregularity has been associated with lower bone mass in studies of female athletes who had low BMI and extensive training [123-126]. However these results may not directly apply to today's population of young women with a higher prevalence of overweight and obesity. Recent studies have suggested that menstrual irregularity is associated with higher levels of androgens in a population-based sample of premenopausal women [127, 128], while in female elite athletes oligomenorrhea was associated with higher bone mass and PCOS [129]. These results may in turn suggest a positive association between menstrual irregularity and bone mass. However, few studies have examined the associations between menstrual irregularity, androgen and bone mass in population-based sample. Therefore, the aim of this cross-sectional study was to describe the association between menstrual pattern, testosterone, sex hormone binding globulin (SHBG) and bone mass measured by quantitative ultrasound (QUS) in a population-based sample of young women.

6.2 Materials and Methods

6.2.1 *Subjects*

This study utilized data from the Childhood Determinants of Adult Health (CDAH) study, a 20-year follow-up of children who were randomly selected for the 1985 Australian Schools Health and Fitness Survey (ASHFS) at age 7-15 years. Details of the 1985 sampling strategy have been described elsewhere [43]. During follow-up (2004-2006) a total of 2410 subjects aged 26-36 years (48% male, 52% female), completed questionnaires and attended one of 34 study clinics in major cities and regional centres around Australia for extensive physical measurements including anthropometric measures, quantitative ultrasound and blood biochemistry.

Eligible subjects for this study were women who had completed study questionnaires, attended study clinics, were not currently taking oral contraceptives, and not pregnant or breast feeding at the time of clinic attendance. Of the 1260 women who attended clinics, 82 were currently pregnant; 453 were currently taking hormonal contraceptives (including combined and progestin only contraceptives and progestin releasing intrauterine devices) or medications such as hormonal agents for PCOS or in vitro fertilization (IVF); 44 did not provide their menstrual cycle characteristics; and 299 had missing QUS measurements due to the machine being out of service, leaving a total of 382 participants for this analysis. All participants provided written informed consent and the study was approved by the Southern Tasmania Health and Medical Human Research Ethics Committee.

6.2.2 *Assessment of menstrual cycle characteristics*

Menstrual cycle characteristics were obtained by written questionnaire. We defined the menstrual cycle as the time from the first day of one period to the first day of the next. The question was then asked: “thinking about the most recent time when you were having periods and were not using hormonal contraceptives (e.g., the pill) and were

not pregnant or breast feeding: would you describe your period as very regular, fairly regular, irregular or very irregular [56]. To simplify analyses, menstrual cycle pattern was defined as regular if women described their periods as very or fairly regular, and irregular if irregular or very irregular.

6.2.3 *Hormone measurements*

Blood samples (32ml) were collected from participants after an overnight fast. Plasma insulin was initially measured by a microparticle enzyme immunoassay kit (AxSYM; Abbot Laboratories, Abbot Park, IL) and later, following a change in the choice of kit by the testing laboratory, by electrochemiluminescence immunoassay (Elecsys Modular Analytics E170; Roche Diagnostics, Mannheim, Switzerland). Due to this change in assay methodology, insulin levels from participants' samples (N = 258) assayed using the first methodology were corrected to levels in samples assayed using the second methodology (as per correction factor equation of the laboratory). Total testosterone concentrations were estimated by radioimmunoassay (RIA) developed by Repromed Laboratory (Dulwich, South Australia), which is sensitive for lower levels of testosterone down to 347 pmol/L. SHBG was measured using a non-competitive liquid-phase immunoradiometric assay (SHBG-IRMA kit, Orion Diagnostica, Espoo, Finland). For testosterone, the intra- and inter- assay coefficients of variation (CV) were 6% at 1 nmol/l and 15%, respectively. For SHBG, the intra- and inter- assay CV were 15.4%, and 2.0-8.6% respectively. FAI was calculated as: testosterone (nmol/L) * 100 / SHBG (nmol/L).

6.2.4 *Anthropometric measurements*

Anthropometric measures were taken at study clinics by trained personnel. Height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively, and body mass index (BMI) was calculated as the ratio of weight (kg) to height (m) squared (kg/m^2). Waist to hip ratio (WHR) was calculated by dividing waist by hip circumference measured to the nearest 0.1 cm.

6.2.5 *Quantitative ultrasound measurements*

All participants had calcaneal QUS measured by a single Sahara Clinical Bone Sonometer (Hologic Inc., MA, USA). The ultrasound system consists of two sound transducers mounted coaxially on a motorized calliper with one transducer acts as an emitter and the other as a receiver. This makes direct contact with the heel through elastomer pads and an ultrasonic coupling gel. The lower part of the dominant leg was placed immobilized during measurement and the proper leg angle set by a positioning aid. Broadband ultrasound attenuation (BUA, dB/MHz) and speed of sound (SOS, m/s) were measured at a fixed region in the mid-calcaneus. The quantitative ultrasound index (QUI) was derived from SOS and BUA using the equation: $QUI = 0.41 \times (BUA + SOS) - 571$ [130]. Quality assurance was performed daily by calibrating the device on a dedicated phantom supplied by the manufacturer. The coefficient of variation (CV) for QUS measures was 1%.

6.2.6 *Assessment of other covariates*

Demographic and lifestyle information was obtained by questionnaire. Smoking status was classified as non-smoker or current smoker. Women were classified as nulliparous if they had not had a live birth or parous if they had had at least one live birth. Physical activity was assessed as we have described previously [81] by the International Physical Activity Questionnaire (IPAQ) and as mean steps per day measured by Yamaz pedometer worn for seven days. The questionnaire asked whether participants had ever been told by a doctor that they had polycystic ovaries (PCO) or polycystic ovary syndrome (PCOS).

6.2.7 *Statistical analysis*

The t-test for unequal variance was used to assess differences in normally-distributed continuous characteristics in this study across categorized menstrual cycle

pattern (regular and irregular), the Wilcoxon rank-sum test was used for skewed continuous variables and the two-sample test of proportions was used for dichotomised

covariates. Spearman correlations were used to estimate crude associations between continuous exposures and QUS. Multivariable linear regression analysis was used to explore the association between all QUS measures and menstrual cycle pattern and hormonal factors (testosterone, SHBG and FAI). QUS parameters and hormonal factors were logarithmically transformed to fit linear regression models and standardised to enable direct comparisons between BUA, SOS and QUI. The coefficients represent a change of one standard deviation. Age, BMI and smoking were considered as confounders or covariates of importance, and adjusted for in multivariable models where appropriate. All statistical analyses were performed on intercooled Stata 9.2 for windows (Statacorp, Texas, USA).

6.3 Results

In this relatively young study sample with age range of 26-36 years (mean age 32 years; mean BMI 24.8 kg/m²), most women were married or living as married (61.7%) and were non-smokers (85.7%). Over half the women had had at least one livebirth (53.7%). Only 11% of participants had irregular menstrual cycles. Very few women sampled reported having ever been told by a doctor that they had PCO or PCOS (n= 9 with irregular cycles, n=9 with regular cycles). Women currently taking hormonal medication for PCOS (n=2) were ineligible for this analysis and excluded. Compared with other women in this study, women who were excluded due to current use of hormonal contraceptives or hormonal medications, were slightly younger (30.7 vs 31.5 years) and more likely to have a history of menstrual irregularity (21.6% vs 10.7%), but there was no significant difference in BMI between the groups (25.0 kg/m² vs 24.8 kg/m², p=0.54) and similar proportions were current smokers (13.2% vs 14.3%, p=0.69).

In the unadjusted analysis, women with irregular cycles had higher FAI, SOS and QUI, and lower SHBG compared with those with regular cycles (Table 6.1). The difference in bone mass between women with regular and irregular cycles is illustrated in Figure 6.1. There were no significant differences in age, body composition measurements, marital status, number of live births, smoking and plasma insulin levels between women with irregular cycles and regular cycles. However, all anthropometric measures including BMI, waist circumference and waist to hip ratio were significantly associated with BUA (p<0.05-0.01) (Table 6.2) as were hormonal factors with a positive association between testosterone and FAI, and a negative association with SHBG (all p<0.01) (data not shown).

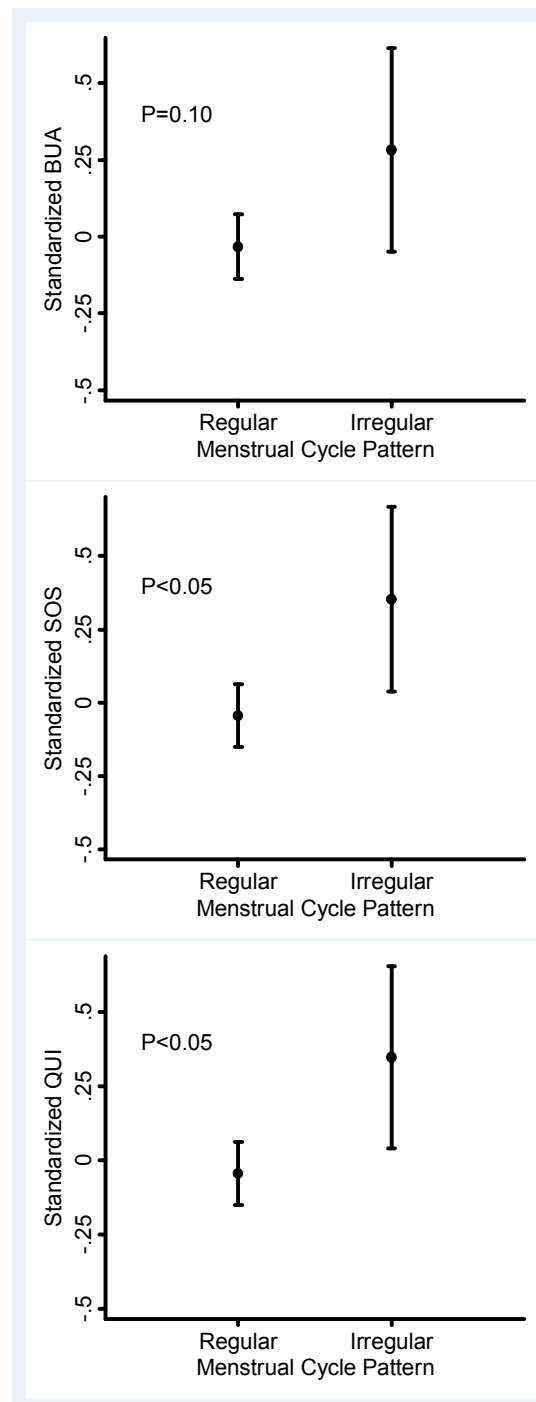
Table 6.1. Characteristics of participants by menstrual cycle pattern

Characteristics	Regular cycle N=341	Irregular cycle N=41	<i>P</i> values
Age (year)^a	31.6 (2.6)	30.9 (2.4)	0.11
Anthropometrics^a			
Body mass index (kg/m ²)	24.8 (5.0)	24.5 (5.9)	0.77
Waist circumference (cm)	78.8 (11.4)	77.8 (13.3)	0.65
Waist-hip ratio	0.757(0.062)	0.760 (0.061)	0.75
Married^b %	62	56	0.44
Parous^b %	54	49	0.50
Current smoker^b %	15	13	0.73
Physical activity (PA)^c			
Pedometer steps (per day)	8331 (6758-10623)	8325 (6715-10992)	0.68
Leisure time PA (min/ week)	120 (0-219) 680 (385-1030)	66 (0-180) 560 (300-970)	0.24 0.27
Hormonal factors^c			
Testosterone (nmol/L)	1.50 (1.22-1.86)	1.53 (1.35-1.89)	0.24
SHBG (nmol/L)	50.3 (35.8-68.0)	43.6 (30.1-54.6)	0.01
Free androgen index	2.94 (2.00-4.47)	4.22 (2.60-6.24)	0.01
Insulin (mU/l)	3.9(5.3-7.5)	3.5 (4.9-7.9)	0.85
Quantitative ultrasound^c			
BUA (dB/MHz)	73.6 (65.4-84.6)	78.7 (67.9-87.1)	0.10
SOS (m/s)	1563 (1543-1587)	1569 (1557-1606)	0.02
QUI (%)	99.5 (88.6-114.1)	106.7 (94.6-121.6)	0.03

^a presented by mean and standard deviation (SD), *P* values derived from t-test for unequal variance.

^b presented by percentage, *P* values derived from test of proportions. ^c presented by median (inter-quarter range), *P* values from Wilcoxon rank-sum test. Bold-face figures denote statistically significant result. SHBG= sex hormone binding globulin. BUA=broadband ultrasound attenuation. SOS=speed of sound. QUI=quantitative ultrasound index.

Figure 6.1. Standardized broadband ultrasound attenuation (BUA), speed of sound (SOS) and quantitative ultrasound index (QUI) for women with regular and irregular cycles



Error bars indicate 95% confidence intervals. SOS, BUA and QUI were logarithmically transformed to fit linear regression models and standardized with mean of zero and standard deviation of one.

There were significant associations between hormonal factors and BUA, SOS and QUI measurements found in both unadjusted correlation analysis (Table 6.2) and adjusted analysis (Table 6.3). Total testosterone and FAI were significantly positively associated with QUS measurements, while SHBG was negatively associated with QUS measures. These associations remained significant after adjustment for confounders with the exception of SHBG and BUA. Further adjustment for history of PCO/PCOS did not change the significant associations. The association between hormonal factors and QUS measures is further illustrated in Figure 6.2, where there were dose-response relationships between BUA measures and hormonal factors, especially for FAI.

Table 6.2. Spearman correlation coefficients (r) for associations of quantitative ultrasound measures and continuous variables in the study

Characteristics	BUA		SOS		QUI	
	r	P	r	P	r	P
Anthropometrics						
Body mass index	0.17	<0.01	-0.01	0.79	0.05	0.35
Waist circumference	0.17	<0.01	-0.01	0.86	0.05	0.32
Waist-hip-ratio	0.14	0.01	0.03	0.57	0.07	0.16
Hormonal factors						
Testosterone	0.14	0.01	0.09	0.08	0.11	0.04
SHBG	-0.16	<0.01	-0.14	0.01	-0.16	<0.01
Free androgen index	0.21	<0.001	0.16	<0.01	0.19	<0.001

SHBG=sex hormone-binding globulin. BUA=broadband ultrasound attenuation.

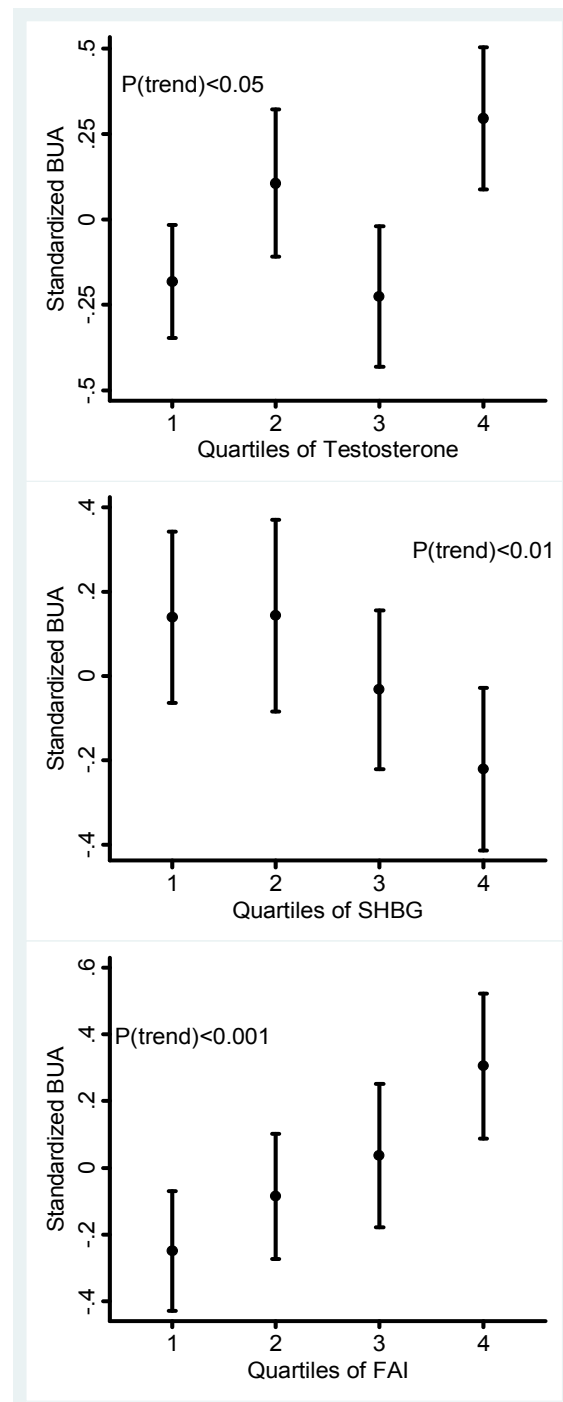
SOS=speed of sound. QUI=quantitative ultrasound index. Bold-face figures denote statistically significant association (at $P<0.05$).

Table 6.3. Association between quantitative ultrasound (QUS) parameters and hormonal factors

	Unadjusted model	Adjusted model	
	(n=382) β (95% CI)	Model 1 (n=358) β (95% CI)	Model 2 (n=358) β (95% CI)
BUA			
Testosterone	0.15 (0.04, 0.24)	0.14 (0.04, 0.25)	0.13 (0.03, 0.24)
SHBG	-0.14 (-0.24, -0.04)	-0.07 (-0.19, 0.05)	-0.05 (-0.17, 0.06)
FAI	0.18 (0.08, 0.28)	0.14 (0.03, 0.26)	0.13 (0.01, 0.24)
SOS			
Testosterone	0.11 (0.01, 0.21)	0.13 (0.03, 0.24)	0.12 (0.02, 0.23)
SHBG	-0.13 (-0.23, -0.03)	-0.16 (-0.27, -0.04)	-0.14 (-0.26, -0.02)
FAI	0.16 (0.06, 0.26)	0.20 (0.08, 0.31)	0.18 (0.06, 0.29)
QUI			
Testosterone	0.12 (0.02, 0.22)	0.14 (0.03, 0.24)	0.13 (0.02, 0.23)
SHBG	-0.14 (-0.24, -0.04)	-0.13 (-0.25, -0.01)	-0.11 (-0.23, 0.01)
FAI	0.17(0.07, 0.27)	0.18 (0.07, 0.29)	0.16 (0.05, 0.28)

Model 1 adjusted for age, BMI and smoking. Model 2 further adjusted for menstrual cycle pattern. QUS measures and hormonal parameters were log transformed before converted into standardized variables. BUA=broadband ultrasound attenuation. SOS=speed of sound. QUI=quantitative ultrasound index. SHBG=sex hormone-binding globulin. FAI= free androgen index. β =standardised beta coefficients. Bold-face figures denote statistically significant association (at $P<0.05$).

Figure 6.2. Standardized broadband ultrasound attenuation (BUA), across quartiles of testosterone, sex hormone-binding globulin (SHBG) and free androgen index (FAI)



Error bars indicate 95% confidence intervals. BUA and hormonal factors were logarithmically transformed to fit linear regression models and standardized with a mean of zero and standard deviation of one. P(trend)=P value for trend.

The associations between irregular cycles and QUS measures are presented in Table 6.4. In multivariable regression analysis, irregular cycles remained significant associations with SOS and QUI ($\beta = 0.38$ for both, $p < 0.05$), with a consistent trend for BUA ($\beta = 0.31$, $p = 0.06$) after adjustment for confounders. The strength of the associations was moderately attenuated when further adjusted for hormonal factors (testosterone, SHBG and FAI separately), with maximally a 27% decrease in the coefficient when further adjusted for FAI, and a lesser effect when adjusted for total levels of testosterone. However women with irregular cycles still had an increase of 0.30-0.31 in the standardized log-transformed SOS and QUI and this corresponds to a maximum 6% increase in the mean QUS measurements.

We recalculated free testosterone (FT) in the equation by taking into account the laws of mass action and using a stable mean of albumin concentration (assumed to be 43g/l), and found that the associations between menstrual irregularity and bone mass were nearly the same when adjusted for FAI and FT. Further adjustment for a history of ever been told by a doctor that they had PCO or PCOS made the associations a little stronger. When irregular and very irregular cycles were examined separately, the adjusted associations were similar in magnitude but were not statistically significant, likely due to lower power ($n = 22$ for irregular, $n = 19$ for very irregular; data not shown).

Table 6.4. Association between quantitative ultrasound (QUS) measures and menstrual irregularity adjusted by each hormonal factor separately

	Adjusted model *	Adjusted model + testosterone	Adjusted model + SHBG	Adjusted model +FAI
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
BUA				
Regular cycle	Ref	Ref	Ref	Ref
Irregular cycle	0.31 (-0.01, 0.64)	0.29 (-0.04, 0.61)	0.28 (-0.05, 0.61)	0.25 (-0.07, 0.58)
SOS				
Regular cycle	Ref	Ref	Ref	Ref
Irregular cycle	0.38 (0.05, 0.71)	0.36 (0.03, 0.68)	0.31 (-0.02, 0.64)	0.30 (-0.03, 0.63)
QUI				
Regular cycle	Ref	Ref	Ref	Ref
Irregular cycle	0.38 (0.05, 0.71)	0.36 (0.03, 0.68)	0.32 (-0.01, 0.65)	0.31 (-0.02, 0.63)

*Adjusted model controlled for age and BMI. QUS measures were log transformed before converted into standardized values. BUA=broadband ultrasound attenuation. SOS=speed of sound. QUI=quantitative ultrasound index. SHBG=sex hormone-binding globulin. FAI=free androgen index. β =standardised beta coefficients. Bold-face figures denote statistically significant association (at $P<0.05$).

6.4 Discussion

In this cross-sectional study of a population-based sample of premenopausal women (37 % overweight or obese), an irregular menstrual cycle was associated with higher quantitative ultrasound measurements including BUA, SOS and QUI. This association was independent of age and BMI but was moderately reduced when further adjusted for hormonal factors, particularly for SHBG and FAI, suggesting that these hormonal factors partially mediate the associations.

Studies have reported a negative association between oligo/amenorrhea and bone mineral density [123-126, 131] in female athletes with low BMI. The bone loss was attributed to low levels of estrogen and/or chronic energy deficiency caused by restricted food intake and extensive training [132]. However, these conditions are not common in the general community and these results may not be applicable to young women affected by today's obesity epidemic. In fact, in this population-based sample of young women, the mean BMI was 24.8 kg/m² which is close to the lower cut-point of overweight (25-30 kg/m²) based on the WHO categorization [133]. We found that irregular cycles were associated with higher bone mass and this was partially mediated by SHBG and FAI. This is consistent with other studies' findings that bone mass was associated with higher levels of androgens and lower levels of SHBG [134-136]. Furthermore we found a significant dose-response relationship between hormonal factors and bone mass, especially for FAI.

Few studies have examined bone mass and the association with menstrual irregularity and androgens in population-based samples of premenopausal women though positive associations between amenorrhea and bone mass were reported in women with hirsutism and PCOS – conditions with excess androgen. Endogenous androgens were associated with menstrual irregularity in population-based [127, 128] and clinical samples [137], and bone mass in premenopausal women [134, 135, 138], but these associations were investigated separately. An important strength of our study, therefore, was its population-based sample and ability to examine the role of hormonal factors.

The mechanism for the association between menstrual irregularity, hormonal factors and bone mass is not clear. However the association between menstrual irregularity and bone mass had a moderate reduction in strength and lost significance when further adjusted for either SHBG or FAI separately. This suggests that bone mass could be influenced by SHBG through the regulation of free testosterone levels. Our result is consistent with a prospective study which examined 231 women aged 32-77 years over 2-8 years period and found, in addition to age and weight, SHBG was a strong independent predictor of BMD [138]. A recent editorial discussed a potential dual role for SHBG (inhibitor or facilitator) in regulating sex hormone action [139] following a genetic study of polymorphisms in the SHBG gene promoter which found that serum levels of SHBG were positively associated with BMD in elderly men [140]. However our result favours the free hormone hypothesis that SHBG inhibits sex hormone bioavailability, rather than augments sex hormone action.

There were several limitations which should be considered when interpreting these findings. First, menstrual cycle characteristics were obtained by self-administered questionnaire which did not include specific criteria for women to assess their cycle regularity. This may have resulted in misclassification. However, it is unlikely that this error would be differential with respect to bone mass or hormonal factors since both QUS and hormonal factors were measured at the same time that women reported their menstrual cycles. Therefore the association between irregular cycles and bone mass may be underestimated in our study. Second, we did not have dual-energy x-ray absorptiometry (DXA) measurement of BMD; however QUS results correlate well with BMD measured by DXA at the heel [53] and predict fracture risk similarly to DXA [54]. This study was conducted in community centres across Australia so DXA measurements were not feasible. Furthermore, use of a portable QUS machine avoided standardisation issues which could have been a major problem had we used multiple DXA instruments at different sites. Third, we did not assess endogenous estrogens which show substantial variation during the menstrual cycle. Clearly, estrogens play a major role in regulating bone mass in both males and females. Previous studies examining both estrogens and androgens in premenopausal

women found that androgen but not estrogen was associated with bone mass [134, 138] suggesting premenopausal estrogen levels may be sufficient in most women. Due to logistic issues, the blood sample collection was not timed with the menstrual cycle possibly introducing some measurement error as testosterone varies with the menstrual cycle [141]. Another limitation is that some young women with irregular cycles may have had undiagnosed PCOS. However, this sample of young women had testosterone levels typically within the normal range. Exclusion of eight women who had testosterone levels higher than 2.9 nmol/l (the upper limit of the laboratory's reference range for premenopausal women with normal menstrual cycles and no evidence of polycystic ovaries on ultrasound) or 24 women who had SHBG levels less than 24 nmol/l (the lowest levels for follicular phase for premenopausal women) made little difference to the findings. In addition, adjustment for a history of ever being told by a doctor they had PCO or PCOS did not decrease the associations. Further, the relatively low percentage of eligible participants with complete data may limit the generalizability of our findings to the general population. Finally, we cannot be certain of the causal direction of the associations observed due to the cross-sectional design of this study. Thus, a longitudinal study is necessary to confirm these findings.

6.5 Conclusions

Irregular menstrual cycles were associated with higher bone mass in this population-based sample of premenopausal women suggesting menstrual disturbance should continue to be evaluated but may be less harmful for bone mass than previously believed. The association between menstrual irregularity and bone mass was partially mediated by markers of androgen status especially free testosterone.

Chapter 7

The Associations between Parity, Other Reproductive Factors and Cartilage in Women Aged 50-80 Years

Published in the

Osteoarthritis and Cartilage. 2011, 19 (11):1307-1313

**Chapter 7 has been removed due to copyright or
proprietary reasons**

Chapter 8

The Association between Parity and Knee Cartilage in Women

Aged 31 to 41 Years

Accepted for publication by the

Rheumatology (Id:KES201)

8.1 Introduction

Osteoarthritis (OA) is the most common joint disorders and the leading cause of pain, loss of function and disability in Australia [1]. Women are at higher risk of OA with higher prevalence and incidence, and higher rate of loss of cartilage volume than males [22, 165-167]. While the mechanism underlying the gender differences is not fully understood, studies suggest some reasons for this may include less cartilage development during childhood, a higher rate of cartilage loss after menopause and greater worsening of cartilage defects over time [145]. Receptors of sex hormones including estrogen and progesterone have been found in human cartilage [161, 162] indicating cartilage can respond to sex hormones. However, findings on the effects of sex hormones on OA are still inconclusive [40, 149, 161] which is most likely due to variations in study characteristics, definitions of OA and sites of the disease. In addition, body mass index (BMI) appears to be more strongly associated with both cartilage defects and tibial bone area in women [145] suggesting they are more susceptible to a raised BMI. Reproductive factors, particularly parity, are associated with changes in sex hormones and frequently with body weight [146, 147] and thus may result in the development and progression of OA in women. However, few studies have examined the associations between parity and OA [40, 148-154].

Recently, we reported that there were no associations between parity, other reproductive factors and radiographic OA measured by X-ray in a population-based sample of older women [168], but parity was significantly associated with knee cartilage volume and patella chondral defects measured by magnetic resonance imaging (MRI) suggesting MRI is more sensitive than radiographic assessment [23]. It is uncertain if these reflected long term behaviour changes or were due to long-lasting effects of pregnancy itself as there have been no studies that have investigated the associations between parity and cartilage measures in young women. The aim of this study, therefore, was to describe the associations between parity, knee cartilage volume and cartilage defects in a sample of young women.

8.2 Materials and methods

8.2.1 *Subjects*

This study utilized data from CDAH Knee Cartilage Study which is a follow-up study on a sub-sample of participants who completed the Childhood Determinants of Adult Health (CDAH) study in 2004 to 2006. The CDAH study is a 20-year follow-up of children who participated in 1985 Australian Schools Health and Fitness Survey (ASHFS) at age 7-15years. The CDAH knee Cartilage study occurred from April 2008 to December 2010 by mailing out invitation letters to CDAH participants (n=764) residing in metropolitan Melbourne or Sydney, Australia. Women who agreed to participate were asked to complete short computer assisted telephone interviews (CATIs) and eligible participants were requested to have an MRI scan at either Epworth Hospital in Melbourne or North Shore Private Hospital in Sydney. Exclusion criteria for this study included being pregnant, having had diseases which may affect knee cartilage such as rheumatoid arthritis, and having contraindication for MRI such as metal sutures (iron filing) in their knees or in other parts of their bodies. Women who had no MRI scan were further excluded from the analyses. Reasons for refusing MRI scan included the long distance needed to travel for MRI, work and family commitments, moving interstate, and claustrophobia.

This study was approved by the Southern Tasmania Health and Medical Human Research Ethics Committee and all participants provided written informed consent.

8.2.2 *Assessment of parity*

Information on parity was available from a second follow-up survey of the CDAH cohort (CDAH-2) which collected data by postal or online questionnaire, or telephone interview during 2009-2011. The numbers of biological children and date of birth were recorded and births were counted only if these occurred before knee MRI was performed.

Parity was then defined based on the number of births (twins only counted as singular). Other reproductive factors were also assessed by questionnaire including age at menarche and current use of oral contraceptive (OC). OC use was categorized as no-current hormonal contraceptive use, current OC use and current progesterone-only contraceptive use.

8.2.3 *Measurement of MRI*

Each participant had an MRI scan of the dominant knee (defined as the lower limb from which the subject stepped off when initiating gait). Two hospitals were involved in the scans but the same type of machine (General Electric Medical Systems, Milwaukee, WI, USA) was employed. Knees were imaged in the sagittal plane on a whole body magnetic resonance unit with use of a commercial transmit-receive extremity coil. The following image sequence was used: a T1-weighted fat saturation 3D gradient recall acquisition in the steady state; flip angle 55 degrees; repetition time 58 msec; echo time 12 msec; field of view 16 cm; 60 partitions; 512 x 512 matrix; acquisition time 11 min 56 sec; one acquisition. Sagittal images were obtained at a partition thickness of 1.5 mm and an in-plane resolution of 0.31 x 0.31 (512 x 512 pixels). Individual plates of cartilage volume (medial tibial, lateral tibial and patella) were isolated from the total volume by manually drawing disarticulation contours around the cartilage boundaries on a section by section basis. These data were then re-sampled by means of bilinear and cubic interpolation (area of 312 μm and thickness of 1.5 mm, continuous sections) for the final 3D rendering. The coefficients of variation (CVs) for cartilage volume measures were 2.1-2.6% [23].

Seven subjects had MRI scans for cartilage volume and bone area performed at both hospitals and data from these scans were used to correct all readings for bias. Bland-Altman plots were used to examine differences between hospitals and determine the nature of any observed bias. Uniform biases were identified for medial and lateral tibial cartilage volume, and remedied by applying a difference correction based on the mean difference.

Value dependent bias (patella cartilage volume) was remedied by applying corrections using the slope and intercept from a fitted linear regression model. Patellar-tibial cartilage volume was calculated by summing cartilage volumes at three sites (patellar, medial tibial and lateral tibial) together.

Cartilage defects on a 0-4 scale were graded at the medial and lateral tibial, medial and lateral femoral and patella sites as follows [69, 70]: grade 0=normal cartilage; grade 1=focal blistering and intracartilaginous low-signal intensity area with an intact surface and bottom; grade 2=irregularity on the surface or bottom and loss of thickness of <50%; grade 3=deep ulceration with loss of thickness >50%; grade 4=full-thickness chondral wear with exposure of subchondral bone. A cartilage defect had to be present in at least 2 consecutive slices and was defined as a score of ≥ 2 at any site measured at the knee (within that compartment). Intra-observer reliability expressed as intraclass correlation coefficient (ICC) was 0.89-0.94, and inter-observer reliability was 0.85-0.93 [71].

The area of medial and lateral tibial plateau bone was measured manually on the three reformatted images closest to tibial cartilage in the axial plane which is transformed from the sagittal images as describe in the previous studies (18, 19, 21). An average of these two areas will be used as an estimate of the tibial plateau bone area. The CVs for these measures were 2.2-2.6% [23].

One reader assessed cartilage measures and was blinded to parity status of the women.

8.2.4 *Assessment of covariates*

Weight was measured to the nearest 0.1kg using a single pair of electronic scales (Delta Model 707; Seca, Hamburg, Germany), with shoes, socks, and bulky clothing removed. Height was measured by Leicester Height Measure (Invicta, Leicester, UK) with

shoes removed. Body mass index (BMI) was calculated based on the measured weight on height (kg/m^2).

Smoking (ever smoker or never smoker) and current employment status (full-time employment, part-time employment and not in workforce) was assessed by the computer assisted telephone interview (CATI). The highest education level achieved (school only, diploma/vocational training or university education) was assessed by the self-administered questionnaire (CDAH-2). Physical activity was assessed by the CATI using the short international physical activity questionnaire (IPAQ) [60]. The IPAQ-short considered three levels of physical activities including vigorous, moderate and walk activities that participants performed for at least 10 minutes in the last seven days, and recorded frequency, duration and intensity of physical activity. Total physical activity was calculated as a score using the equation of Metabolic Equivalent Task (MET, minute/week) based on the time spent over the 7 days and types of physical activities including vigorous, moderate and walking assessed from the IPAQ short questionnaire [60]. A history of knee injury was also assessed by the CATI.

Knee pain was assessed using Western Ontario and McMaster University index (WOMAC) [74]. Five categories of pain (walking on flat surface, going up/down stairs, pain at night, sitting/lying, and standing upright) were assessed separately with a 10-point scale from 0 (no pain) to 9 (most severe pain) for each category. A total pain score can range from 0 to 45.

8.2.5 Statistical analysis

The t-tests for unequal variance were used to assess differences between parous and nulliparous for continuous variables whereas Chi^2 or Fisher's exact tests were used for categorical variables. Descriptive characteristics were presented as means (standard deviation) or percentages. Univariable and multivariable linear regression analyses were employed to examine the associations between parity and cartilage volume before and after

adjustment for confounders. Log-binomial regression analyses were used to investigate the associations between parity and the prevalence of cartilage defects. Age, BMI, smoking, physical activity, knee injury and bone area were considered as confounders and adjusted for in multivariable models. All statistical analyses were performed on intercooled Stata 10.1 for Windows (Statacorp, Texas, USA).

8.3 Results

Among 764 approached CDAH participants, 529 (69) agreed to participate in the follow-up study and 422 completed CATIs. Of these, 296 participants had an MRI scan (144 women). In this study sample, women were aged 31-41 years with a mean age of 36 years and BMI of 25 kg/m². 61% had a tertiary education and over half (58%) were parous with parity ranging from 1 to 5. There were no significant differences between women who were included in this study and those excluded due to absence of MRI scans in age, employment status, knee injury, knee pain score, and physical activity (all $p>0.05$) but study participants were less likely to have ever been smokers (28% vs 42%, $p=0.04$).

Characteristics of study participants are shown in Table 8.1. Compared with the nulliparous women, parous women were older and slightly leaner, and had a lower rate of knee injury. Parous women were also less likely to achieve high levels of education or have full-time jobs compared with the nullipara. There were no significant differences between the parous and the nulliparous in terms of BMI, age at menarche, current use of oral contraceptives, smoking status, knee pain score, cartilage volume, cartilage defects and bone area.

Table 8.1. Characteristics of participants by parity

	Nulliparous (n=61) Mean (SD)	Parous (n=83) Mean (SD)	<i>P value</i>
Age (years)	35 (0.3)	37 (0.3)	<0.0001
BMI (kg/m ²)	25.8 (0.6)	24.4 (0.4)	0.05
Menarche (Years)	12.7 (0.2)	12.9 (1.3)	0.41
Current use of OC (%)	29.6	26.7	0.75
Ever smoker (%)	25	31	0.43
Knee injury (%)	23	9	0.02
Total PA (MET minute/week)	3104 (336)	2793 (282)	0.47
Knee pain score	1.34 (0.37)	1.66 (0.43)	0.57
Patella-tibial cartilage volume (ml)	5.63 (0.16)	5.67 (0.12)	0.86
Presence of tibiofemoral cartilage defects (%)	12	15	0.40
Presence of patella cartilage defects (%)	10	16	0.22
Total knee bone area (cm ²)	27.9 (0.29)	27.8 (0.28)	0.83
Highest education achieved (%)			0.03
School only	10	18	
Diploma/ vocational training	16	30	
University degree	74	52	
Current employment status (%)			<0.001
Full time	77	19	
Part time	20	42	
Not in workforce	3	39	

Date was presented by mean and standard deviation (SD) except indicated. T-test for unequal variance was used to assess differences between parous and nulliparous for continuous characteristics whereas Chi² or Fisher's exact tests were used for categorical

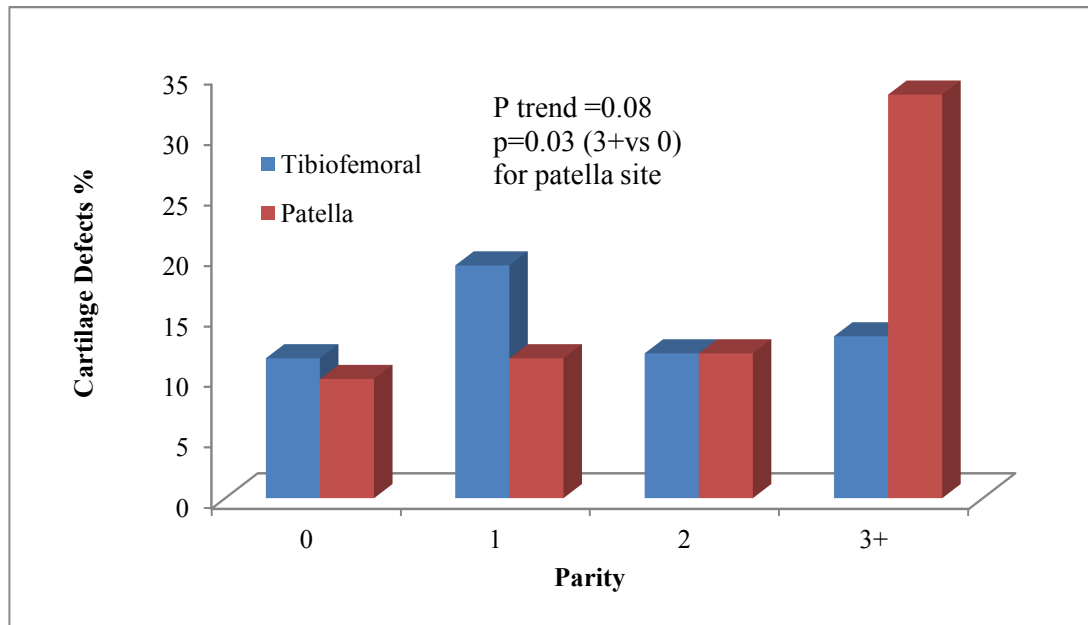
variables. Presence of cartilage defects was defined as any score ≥ 2 at the compartments measured at the knee. BMI: body mass index. OC: oral contraceptive. PA: physical activity. MET: metabolic equivalent task. Bold-face figures denote statistically significant result.

Parity was significantly associated with a higher risk of cartilage defects at the patella site after adjustment for age, BMI, smoking, physical activity, knee injury and bone area, and the risk of cartilage defects increased by 1.52 for each extra birth (Table 8.2). Compared with nulliparous women, parous women also had about twofold greater risk of cartilage defects but this association was not statistically significant (Table 8.2). When examining parity as groups, there was an increased trend of prevalence of the patella cartilage defects with increasing parity and women who had three or more births had significantly increased risk of cartilage defects (Figure 8.1). These associations remained significance after adjustment for confounders (Table 8.2). Further adjustment for highest education level and current employment status did not change the association between parity and cartilage defects (data not shown). There were no significant associations between parity and cartilage defects at the medial and lateral tibiofemoral compartments before and after adjustment for confounders (Table 8.2).

Table 8.2. The association between parity and prevalent cartilage defects

	Unadjusted PR (95% CI)	Adjusted * PR (95% CI)
Patella		
Parity (continuous)	1.36 (0.98, 1.87)	1.52 (1.05, 2.21)
Parous (yes vs no)	1.59 (0.64, 3.97)	1.93 (0.66, 5.65)
Number of children		
0 (n=61)	Ref	Ref
1 (n=26)	1.17 (0.32, 4.36)	1.03 (0.21, 5.03)
2 (n=42)	1.21 (0.39, 3.72)	1.89 (0.46, 7.75)
3+ (n=15)	3.39 (1.19, 9.66)	5.27 (1.39, 20.01)
P vale for trend	0.08	0.03
Medial tibiofemoral		
Parity (continuous)	0.63 (0.36, 1.12)	0.84 (0.52, 1.37)
Parous (yes vs no)	0.74 (0.19, 2.84)	1.37 (0.47, 4.03)
Lateral tibiofemoral		
Parity (continuous)	1.20 (0.80, 1.80)	1.10 (0.69, 1.74)
Parous (yes vs no)	1.65 (0.53, 5.14)	1.50 (0.37, 6.12)

*Adjustment for age, BMI, total physical activity, smoking, knee injury and tibial bone area. Presence of cartilage defects was defined as any score ≥ 2 at the compartments measured at the knee. PR: Prevalence ratio derived from log binomial regression. Bold-face figures denote statistically significant result (at $p < 0.05$).

Figure 8.1. Prevalence of cartilage defects by parity

Presence of cartilage defects was defined as any score ≥ 2 at the compartments measured at the knee.

The association between parity and cartilage volume is shown in Table 8.3. There were no significant associations between parity (as either a continuous or a categorical variable) and cartilage volume measured at patellar-tibial, or any specific individual site, before and after adjustment for confounders.

Table 8.3. The association between parity and patella-tibial cartilage volume

	Unadjusted	Adjusted*
	β (95% CI)	β (95% CI)
Parity (continuous)	-0.01 (-0.17, +0.16)	+0.01 (-0.16, +0.19)
Parous (yes vs no)	+0.03 (-0.34, +0.41)	+0.10 (-0.30, +0.51)
Number of children		
0 (n=61)	Ref	Ref
1 (n=26)	-0.02 (-0.54, +0.50)	+0.004 (-0.51, +0.52)
2 (n=42)	+0.20 (-0.24, +0.65)	+0.30 (-0.18, +0.78)
3+ (n=15)	-0.35 (-1.00, +0.29)	-0.26 (-0.92, +0.40)
P for trend	0.88	0.87

*Adjustment for age, BMI, total physical activity, smoking, knee injury and tibial bone area. β : regression coefficient.

8.4 Discussion

Parity was associated with increased prevalence of cartilage defects primarily at the patellar site in this sample of young women. Number of live births was also associated with increased risk of having patella cartilage defects with three or more births having the highest risk of prevalent cartilage defects. These results are consistent with our recent findings in older women, suggesting that parity may play a direct role in the development of OA.

Several studies have explored the associations between parity and clinical and/or radiographic OA, or risk of joint replacement [40, 148-154], but none of these studies were performed in a sample of young women. Of the six studies were involved in knee and/or hip OA, five studies showed no association with radiographic or symptomatic knee and/or hip OA [148, 150-153], and one studies reported positive associations with risk of knee and hip joint replacement for OA [40]. Different results may be due to variations in the characteristics of study participants, definitions of OA, site of disease measured, or the ability to adjust for confounders. It may also be due to the lack of sensitivity of radiographic assessments. In ore previous study, we found that parity was associated with cartilage measures assessed by MRI in older women but was not associated with radiographic OA [168].

In contrast with our findings in older women [168], parity was not associated with cartilage volume in this sample of young women. The may reflect the coexistence of cartilage swelling and cartilage loss induced by pregnancy in early years and cartilage volume assessment by MRI is unable to differentiate these. It might also be explained by a smaller sample size in this study and insufficient power to detect similar reductions in cartilage volume. Cartilage defects are regarded as early markers of cartilage damage, which precede cartilage loss [145]. Thus, in a younger age group, parity may be associated with cartilage defects but not yet with loss of cartilage volume, which has been confirmed by the current study in older women.

There was a variation between sites with parity being associated with patella but not tibial or femoral cartilage defects. This is largely unexplained. It may reflect site differences of the effect and/or initial cartilage damage site as the prevalence of cartilage defects was higher in the patella site (13%) than tibial (8%) or femoral (7%) sites in this sample.

The mechanism underlying the association between parity and cartilage defect is not clear. There are several explanations for the association including increased joint load due to weight gain, increased levels of sex hormones such as estrogen and progesterone, and change in lifestyle behaviour including increased household chores or a reduction in recreational physical activity during the period of pregnancy and breastfeeding. Pregnant women in the third trimester produced larger knee moment during sit-to-stand transition from lower chair height [169] and increased loading on knee joint particularly after three or more births can produce unwanted effects on knee cartilage.

Increased sex hormone levels during pregnancy would have a protective effect on cartilage [170]. However, we found the opposite effect suggesting increased sex hormones may not be responsible for the adverse effects of parity on cartilage. The levels of relaxin increase during a pregnancy leading to pelvic and more widespread joint laxity, which may be potential explanatory mechanism [171]. High parity may be associated with lower socioeconomic status and more physical work, and thus increased cartilage damage. However, highest education level achieved and current occupation status were not associated with either cartilage volume or cartilage defects and further adjustment for these factors did not change the associations between parity and cartilage defects, suggesting that socioeconomic status and physical work did not explain the association between parity and cartilage defects. Furthermore, the presence of knee injury was lower in parous than nulliparous women, and adjustment for knee injury did not change the results, suggesting trauma was not an explanation.

This study has some potential limitations. Study factors were assessed by questionnaires which may result in measurement error and misclassification. However, recall of biological children by a computer assisted telephone interview would be expected to be accurate [172]. It is unlikely that any misclassification would be differential with respect to cartilage or reproductive factors since they were measured at the same time. Thus the association observed could be underestimated. Although this study is cross-sectional in nature, there was a temporal and dose-dependent association between parity and cartilage defects suggesting a biologically plausible association.

In conclusion, parity was associated with higher knee cartilage defects primarily at the patellar site in this sample of young women. This association was apparent for women having three or more births suggesting a possible adverse effect of higher parity on knee cartilage.

Chapter 9

Summary and Future Directions

9.1 Summary

Osteoporosis and osteoarthritis (OA) are the most common musculoskeletal disorders affecting older populations. The consequences of these diseases are pain, fractures and disability which place significant financial and personal burdens on the community. Female sex has been a known risk factor for both diseases with a higher prevalence and incidence in women than age-matched men. While sex hormones may be one of the possible explanations of the gender differences, the mechanism by which these hormones affect joint health remains unclear. Reproductive factors may also contribute to the gender differences but findings from studies of radiographic OA and bone mass are not conclusive. There have been few studies so far that have investigated the association between reproductive history and cartilage measured by Magnetic resonance imaging (MRI) which has been shown to be a more sensitive measure of cartilage than radiography. In this thesis, we have examined several reproductive factors and their associations with bone mass, MRI determined cartilage and radiographic OA in population-based samples of both young and older women. Novel and important findings are presented that improve understanding of the possible roles of reproductive factors in the development of these two chronic diseases.

Chapters 4 and 5 revealed the positive association between use of combined oral contraceptive and bone mass in population-based samples of both young and older women. In young women (26-36 years), multivariable analyses showed that current OC use was associated with higher bone mass (4%) measured by quantitative ultrasound (QUS) with similar effects seen for broadband ultrasound attenuation (BUA), speed of sound (SOS) and quantitative ultrasound index (QUI). Progestogen -only contraceptives appeared to be associated with higher bone mass but these associations were not statistically significant in this small sample (n=43). Consistent with the findings in young women, in older women (50-80 years), ever use of OC was positively associated with BMD measured at the total body (6%) and spine (4%) but not hip after adjustment for confounders. Duration of OC

use was also associated with total body and spine BMD. Further analysis of vertebral deformity revealed a negative association with those who used OC for 5-10 years. While causation cannot be inferred from these cross-sectional analyses, the consistent findings from both young and older women suggest that OC use may have long-term beneficial effects on bone health. Progestogen-only contraceptives appeared to have no deleterious effect on bone mass but this need to be further investigated. These findings may help with decision-making when balancing the advantages and disadvantages of OC use particularly for those who are using or require use of OC.

Chapter 6 presented the first study so far to describe the associations between menstrual irregularity, hormonal factors and bone mass in a population-based sample of young women. Menstrual regularity was positively associated with bone mass assessed by QUS (including SOS and QUI) and free androgen index (FAI) but negatively associated with sex hormone-binding globulin (SHBG). Further adjusted for hormonal factors separately (testosterone, FAI and SHBG), the association between menstrual irregularity and bone mass was moderately decreased in strength (maximum 27% decrease when adjusted for FAI) but women with menstrual irregularity still had 4% higher bone mass than those who had regular menstrual cycles indicating an independent association between menstrual irregularity and bone mass. It appears that bone mass in young women may be influenced by SHBG through regulating free androgen levels but androgen status may not be the only pathway involved in the association between irregular cycle and bone mass. These results suggest that menstrual disturbance may not be as harmful for bone mass as previously believed but the mechanism underlying the associations needs to be further investigated.

Chapters 7 and 8 represented novel studies of the associations between parity and cartilage measures in both young (age 31-41 years) and older women (aged 50-80 years). In young women, there were no significant associations between parity and MRI determined cartilage volume measured at total knee or any specific individual site. However, parous women had significantly higher risk of cartilage defects measured in the

compartment of total knee and patella compared with nulliparous women. Similarly, the risk of cartilage defects increased with each extra birth and women who had three or more births had significantly greater risk of cartilage defects than nulliparous counterparts. The conflicting results between cartilage volume and cartilage defects in young women may reflect the coexistence of cartilage swelling and cartilage loss in the early stage of the disease in such young women and cartilage volume assessment may be insensitive to these early changes in cartilage. In fact, in older women, parity was significantly associated with lower knee cartilage volume and higher patella cartilage defects. In addition, parity was dose-dependently associated with decreasing cartilage volume in both the tibial compartment and total knee suggesting a biologically plausible association. Analyses of parity and radiographic OA presented a consistent but non-significant increase in JSN and osteophytes for parous women possibly because radiography is less sensitive than MRI. There were no associations between uses of HRT and/or OC, cartilage measures or radiographic change suggesting exogenous hormones may not play an important role in cartilage changes in this group of women. Findings from both young and older women consistently suggest parity may play an independent role in the development of OA, and the parity related knee cartilage damage may start as early as in women's thirties.

In conclusion, these cross-sectional analyses of population-based samples of both young and older women showed that use of OC was associated with higher bone mass suggesting a protective effect of OC use on bone health. In young women, menstrual irregularity was associated with alterations of sex hormones but may not be as harmful for bone mass as previously believed. Parity, particularly higher parity, was associated with higher cartilage defects in young women and low cartilage volume in older women indicating a possible effect of childbearing on the development of OA in women.

9.2 Future directions

In this thesis the associations between reproductive factors, bone mass, cartilage and radiographic OA have been described in population-based samples of both young and older women. The novel findings are that parity was associated with higher risk of cartilage defects in young women and lower cartilage volume in older women, and menstrual irregularity was associated with higher bone mass in young women. These findings provide more insight into the gender differences of the two major joint disorders affecting older people in particular osteoarthritis. However the results are derived from the cross-sectional studies which are unable to confirm whether the relationships are causal. Therefore the most important future work would be to perform prospective cohort studies to confirm the associations found from this thesis.

In Chapter 4, we reported a positive association between use of OC and bone mass in a population-based sample of young women suggesting OC use in young women may have a beneficial effect on bone mass. The results however require replication in longitudinal studies in order to confirm a causal relationship. We have examined hormonal contraceptive use by hormonal content (combined estrogen and progestogen or progestogen-only contraceptives) but dose of estrogen contained in OC was not assessed. Assessment of dosage of OC should be the subject of future work as this may be associated with bone mass. The estrogen content in OC has decreased over the years with an intention to decrease related cardiovascular events. However there are concerns regarding whether lower-dose OC provides sufficient estrogen supplementation for bone mass accrual in adolescents and young women as they are more likely to use hormonal contraceptives during a period of peak bone acquisition [173]. There is also evidence that use of OC at a younger age may impact bone mass acquisition [78, 174]. Therefore additional assessments of these related factors are needed in future studies as this information will help determine the optimal dosage and timing of initiation of OC in young women requiring hormonal contraception. Furthermore, this study investigated current OC use but

not past use or duration of use which may also be associated with bone mass as was suggested in our assessment of older women. Thus assessments of past use and duration should be included in future studies.

The association between progestogen-only contraceptive use and bone mass was examined in this study but the association between progestogen-only contraceptive use and bone mass is uncertain due to the small number of women who used these contraceptives. Therefore this association between progestogen-only contraceptive and bone mass needs to be further investigated in a larger sample with more women with progestogen-only contraceptives. Moreover, the progestogen-only contraceptive group combined women using varied types of progestogen-only contraceptives. Previous studies of progestogen-only contraceptive use have shown that use of Depot Medroxyprogesterone Acetate (DMPA) had an adverse effect on bone mass but others may have no effect on BMD [80]. Thus future work should investigate the effect of progestogen-only contraceptive use taking into account the different types of contraceptives used.

Chapter 5 presented positive association between use of OC and bone mineral density in population-based sample of older women that is consistent with the finding in young women. Duration of OC use was also associated with higher BMD. Use of HRT and menopause status were associated with BMD but adjustment for these factors and other confounding factors did not eliminate the positive association suggesting OC use during the reproductive years may have an independent protective effect on bone health. However, this study in older women again did not assess the dosage of estrogen contained in OC, and OC used in the 1960s and 1970s in Australia tended to have higher doses of estrogen (50 mcg of ethinyl estradiol) than OC used in more recent decades (30-40 mcg of ethinyl estradiol). Thus further study needs to investigate the association with bone mass taking into account the different doses.

In Chapter 5, vertebral deformity was also examined. OC use was found to be associated with a reduction of vertebral deformity but only for those women who used OC for 5-10 years. Use of OC for more than 10 years was not associated with more protective effect. This association is largely unexplainable but it may reflect differences in the timing of OC exposure. A previous study has reported that years of OC exposure may be associated with risk of fractures in later life [119]. Therefore in future, examination of years of OC use may help explain the association. Furthermore, future population-based studies would ideally include women with diagnosed osteoporotic fractures to confirm the association between OC use, bone mass and fractures.

Chapter 6 described a positive association between menstrual regularity, and bone mass in young women in contrast to previous findings in female athletes with lower BMI. Our results suggest young women who have irregular cycles may have higher rather than lower bone mass and they are more likely to be overweight due to today's obesity pandemic. Menstrual irregularity was associated with higher FAI and lower SHBG which partially explained the association between menstrual irregularity and bone mass. However, menstrual regularity was generally defined as either regular or irregular cycles, and irregular cycles can be more specifically defined as short or long cycles which may have different implications in terms of sex hormones exposures and bone. Therefore this should be important to examine in future.

Androgen status was assessed by levels of testosterone, SHBG and FAI but levels of endogenous estrogen were not assessed in this study. Estrogen has been known as one of the important determinants in attainment of peak bone mass in teenagers, maintaining balance of bone formation and absorption through life, and preventing bone loss in postmenopausal women. While previous studies examining both estrogens and androgens in premenopausal women found that androgen but not estrogen was associated with bone mass [134, 138], future examination of more extensive endogenous sex hormones (estrogen/progesterone) are needed. Results presented in this thesis were derived from cross-sectional analyses of a population-based sample of young women and only relatively

low percentage of eligible participants had complete data. Thus these findings require replication in future prospective cohort studies with a larger population.

Chapter 7 presented the most novel findings from this thesis. Parity was associated with cartilage volume measured at the total knee and tibial compartment in the population-based sample of older women and the associations between parity and cartilage volume were dose-dependent suggesting parity may play a role in the development of OA. However parity was associated with only patella cartilage defects not tibial or other sites. Cartilage defects and cartilage volume may occur at different stages of the development of OA thus may not necessarily be expected to appear together in the same OA knee compartment, however, the inconsistent results mean that more investigations are needed to confirm the association. We examined multiple confounding factors and adjusted for them in this study but there may still have been uncontrolled confounders. Thus assessments of more extensive potential confounders including knee injury, a major risk factor for knee OA, are recommended in future.

Chapter 8 reported an exploratory study which examined for the first time the association between parity, cartilage volume and cartilage defects in young women. Compared with nulliparous women, parous women had significantly higher risk of cartilage defects measured at the patella site. Similarly, the risk of cartilage defects increased with each extra birth and women who had three or more births had significantly greater risk of cartilage defects than nulliparous counterparts. These results suggest childbearing related cartilage damage may start as early as their thirties. Inconsistently, parity was not associated with cartilage volume measured at total knee or any specific individual site indicating the associations between parity and cartilage measures in young women needs to be further investigated. Given the study results derived from a non-randomly selected and relatively small sample of young women, further studies in randomly-selected women with larger sample size are needed to confirm the association.

Reproductive factors and their association with osteoporosis and OA were assessed separately in this thesis. However, there is some evidence has suggested that these two common diseases may share some risk factors but in different directions, such as, overweight may be a risk factor for OA but protective of osteoporosis [26,27]. Therefore a further investigation of reproductive factors and the associations with bone mass, cartilage volume and cartilage defects simultaneously may provide additional information about how these factors affect OA and osteoporosis and whether the effect directions are opposed.

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Invitation to participate letter

16 February 2005

«first_name » «surname »

«address »

«state » «postcode »

Dear «first_name »

RE: Childhood Determinants of Adult Health

I am writing to invite you to take part in a follow-up study of students who participated in the 1985 Australian Schools Health and Fitness Survey. We believe you participated in the original survey when you were «age » years old and a student at «school_name » in «State_wrds ». This follow-up study, known as the **Childhood Determinants of Adult Health**, is being conducted by a team of researchers based at the Menzies Research Institute in Hobart and collaborators in Melbourne. It has been funded by the National Health & Medical Research Council.

The long-term aim of this follow-up study is to determine the importance of health and fitness in childhood in predicting the risk of heart disease and diabetes in later life. In the short-term, the study will provide important new information on how health and fitness in childhood predict health problems of importance to young adults. The detailed information collected from the 1985 Australian Schools Health and Fitness Survey is unique in Australia and makes this follow-up study one of only four internationally with the capacity to answer these questions.

If you agree to participate in the study, we will first ask you to enroll as a participant by completing the enclosed brief questionnaire about yourself and providing us with information to help us contact you again.

At some time during 2005-2006, you will be asked to attend a clinic for a free health check and, subject to funding, will also be invited again ten years later. The follow-up studies will take similar measures to those taken in 1985 and you will also be asked to complete

questionnaires about your health and lifestyle. Further information about the study is included in the enclosed information brochure. Participation in the study is entirely voluntary and you may withdraw from the study at any time. All information provided for the study will remain confidential and no individual will be identifiable in reports or presentations that arise from the study.

We hope that you will agree to participate in the study. Please return the completed questionnaire and informed consent form in the reply-paid envelope, **as soon as you can**. If you do not wish to participate, please let us know.

Because it is very important that we find as many of the original 1985 survey participants as possible, we may contact you to see if you can help us establish the whereabouts, or new married names, of some of your classmates.

The Australian Electoral Commission (AEC) has provided name, address, gender and age-range information for this medical research study in conformity with sections 91(4A)(e) and 91A(2A)(c) of the *Commonwealth Electoral Act 1918* and Regulation 10 of the Electoral and Referendum Regulations. The AEC has not disclosed particulars of the occupation or dates of birth of any electors registered on the Commonwealth Electoral Roll.

If you have any questions about the study, please contact the Recruitment Co-ordinator, Beverley Curry, on our toll-free telephone number 1800 634 124.

Thank you for your cooperation.

Yours sincerely

Associate Professor Alison Venn

Chief Investigator

Childhood Determinants of Adult Health

Acting Director - Menzies Research Institute



CDAH

What is the Study about?

Heart disease and diabetes are major health problems in Australia and the rest of the world. Much of what we know about the importance of lifestyle factors, such as diet and exercise, and the role of blood pressure and cholesterol levels, comes from research in adults.

Some studies have suggested that the early stages of heart disease and diabetes start to occur in childhood.

We need to find out more about how lifestyle and other risk factors in children and young adults affect their chance of developing heart disease and diabetes in later life.

Lots of valuable information was collected from children in the 1985 Australian Schools Health & Fitness Survey. A follow-up study of the long-term health of these children will help us to answer these important research questions.

In addition, the study will help us to understand how lifestyle and physical measures in childhood affect the health of young adults including respiratory and mental health and women's reproductive health.

Who is being asked to participate?

We hope to invite all 8,484 students who participated in the original 1985 Australian Schools Health & Fitness Survey to be in our follow-up study.

The original participants came from 109 schools around Australia, and were aged 7 to 15, with around 20 students in each age group from each school.

What will the study involve?

If you agree to participate in the study we will ask you to do the following:

- Enrol in the study now by returning your completed questionnaire and consent form and providing your contact details.
- Visit a study clinic in your nearest capital city or a regional centre for the first follow-up, which will be during 2003 – 2005.
- At the study clinic you will be asked to complete questionnaires about your medical history, current health status and lifestyle and have a free health check.
- Be part of the second follow-up ten years later in 2013 – 2015.

What do you mean by 'health check'?

The health check will include measures of your blood pressure, height, weight, waist and hip girths, and collection of blood samples. The blood samples will be used to test your blood cholesterol, sugar levels, certain hormones in women, and genetic risk factors.

We will also measure: your lung function, by getting you to blow into a machine; and your heel bone density and the health of your arteries,

using a simple, painless ultrasound procedure. An exercise bicycle will be used to test your fitness. If there are any tests you are unable to complete or prefer not to complete, we would still value your participation in all other areas.

What will happen next?

If you agree to take part in the study:

- You will receive study newsletters.
- You will be contacted again in a year or two to arrange your clinic visit.

Is the study confidential?

Yes. The information you provide will be treated confidentially.

You will be allocated an identification number so that your information can be stored in computer files without your name.

Identifiable information will not be released to anyone outside the research team and will not be used for any other purpose. You will not be identified in any reports or presentations that arise from the research.

Do I have to participate?

No. Participation is voluntary. Even if you agree to participate now, you will be able to withdraw from the study at any time, and can change your mind between now and the testing period.





CONSENT TO PARTICIPATE & FORWARD RESULTS

_____, of

(First Name)

(Last Name)

(Address)

(Suburb)

(Postcode)

- 1) I have read and understood the information brochure for this study.
- 2) The purpose of the study, the expected length of time the examination will take, and an indication of any discomfort which may be expected, has been explained to me.
- 3) I understand that the clinic visit will involve collection of a blood sample, measurement of my blood pressure, lung function, weight, height, waist, hips and skin folds, ultrasound measures of my heel bone density and blood vessels, and some simple fitness tests.
- 4) I agree that a sample of my blood and DNA may be kept indefinitely for future studies to investigate potential links between blood factors or genes and cardiovascular disease and type 2 diabetes.
- 5) I understand that it is my responsibility, when asked, to inform personnel carrying out the testing if I am pregnant or have any injury or medical condition which may make it unsafe for me to carry out any of the tests.
- 6) I understand that all research data will be treated as confidential and no identifying information about me will be released.
- 7) I agree that the findings of the study may be published provided that I cannot be identified as a subject.
- 8) I understand that some of the results of my assessments will be given to me and/or my doctor if I wish.
- 9) I understand that I am free to withdraw from this study at any time.
- 10) I understand that the study has been approved by the Southern Tasmania Health and Medical Human Research Ethics Committee.
- 11) Any questions that I have asked have been answered to my satisfaction.

Signed _____ Date ____/____/____

Witness _____ Date ____/____/____

Signed _____ Date ____/____/____

Witness _____ Date ____/____/____

To whom would you like your results sent? (Please tick one or both boxes)

To myself ☐ To my doctor* ☐

*If you want your results sent to your doctor, please write the name and address below:

Dr _____
(Name)

(Street) _____

(Suburb) _____ (State) _____

(Postcode) _____

Your Marital Status:	Single	<input type="radio"/>	Separated/Divorced	<input type="radio"/>
	Married	<input type="radio"/>	Widowed	<input type="radio"/>
	De facto	<input type="radio"/>	Other	<input type="radio"/>

Which school were you at in 1985?

What is the highest level of formal education that you have completed?

Primary School ☐
 Grade 7-9 ☐
 Grade 10 ☐
 Grade 11 ☐
 Grade 12 ☐
 Trade Certificate ☐
 Technical College ☐
 Undergraduate university studies ☐
 Postgraduate university studies ☐

What is your current employment status?

Employed full-time ☐
 Employed part-time or casual ☐
 Unemployed ☐
 Home duties ☐
 Student ☐
 Permanently unable to work / Disabled ☐

How tall are you?

cm OR ft in

How much do you weigh?

kg OR st lb

Have you ever been a regular smoker?

Yes ☐ No ☐

(A regular smoker is someone who has smoked at least 7 cigarettes, cigars or pipes every week for at least 3 months)

Are you currently a regular smoker?

Yes ☐ No ☐

In general, would you say your health is:

Excellent ☐ Fair ☐
 Very Good ☐ Poor ☐
 Good ☐

CONTACT 1

[illegible][illegible]

Parent ☐ Grandparent ☐ Brother/Sister ☐ Other relative ☐ Friend ☐ Other ☐

[illegible][illegible][illegible]

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[Home](#)

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[illegible][illegible]

Parent ☐ Grandparent ☐ Brother/Sister ☐ Other relative ☐ Friend ☐ Other ☐

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SECTION D: This section is for **WOMEN ONLY**.

If you are MALE please skip to SECTION E (page 15). The answers to the following questions will help us investigate the influence of hormones on the cardiovascular system.

1. Are you currently using any of the following hormonal contraceptives, even if you are using them for reasons other than contraception?

- ☐ Oral contraceptive pill
- ☐ Minipill (progesterone only pill)
- ☐ Weekly contraceptive patch
- ☐ Progestagen (e.g., Implanon)
- ☐ Progestagen injection (e.g., Depo Provera)
- ☐ Progestin injection (e.g. , Noristerat)
- ☐ Progestin releasing intrauterine device (e.g. , Mirena, Copper T380A)
- ☐ Progestin releasing implant (e.g. , Norplant)
- ☐ Other (please specify)

2. How old were you when you had your first menstrual period?

Years	Months

3. Have you had a hysterectomy; that is, an operation to remove your uterus?

- ☐ No -->Skip to Question 4
- ☐ Yes

IF YES

- 3a) What age were you when you had the hysterectomy?

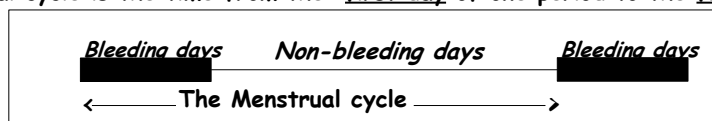
		Years
--	--	-------

- 3b) Were your ovaries removed as well?

- ☐ Yes, both ovaries removed
- ☐ Yes, only one ovary removed
- ☐ No
- ☐ Don't know

SKIP TO Question 5

4. The menstrual cycle is the time from the first day of one period to the first day of the next.



- 4a) How long is your usual menstrual cycle?

In other words, how many days are there from the FIRST DAY OF ONE PERIOD to the FIRST DAY OF THE NEXT?

--	--	--

 Days

- 4b) What is the longest menstrual cycle you have had in the last 12 months? Again count from the FIRST DAY OF ONE PERIOD to the FIRST DAY OF THE NEXT.

--	--	--

 Days

- 4c) What is the shortest menstrual cycle you have had in the last 12 months? Again count from the FIRST DAY OF ONE PERIOD to the FIRST DAY OF THE NEXT.

--	--

 Days

5. Thinking about the most recent time when you were having periods and were NOT using hormonal contraceptives (e.g., the pill) and were not pregnant or breastfeeding:

- 5a) Would you describe your periods as:

- ☐ Very regular
☐ Fairly regular
☐ Irregular
☐ Very irregular

- 5b) How old were you at this time?

That is, at the most recent time when you were having periods and were NOT using hormonal contraceptives (e.g., the pill) and were not pregnant or breastfeeding.

--	--

 Years

- 5c) During this time, approximately how many periods did you have in the space of 12 months?

- ☐ More than 13
☐ 11-13
☐ 6-10
☐ 1-5
☐ None

6. Have you ever seen a doctor because of irregular periods?

☐ No -->Skip to Question 7

☐ Yes

IF YES

6a) How old were you when you first saw your doctor about irregular periods?

Years

6b) Have you ever taken prescribed hormone medications for irregular periods?

☐ Yes ☐ No

6c) Has a doctor ever told you that you have polycystic ovaries or polycystic ovary syndrome?

☐ Yes ☐ No

7. Have you ever seen a doctor because of concern about the amount of hair on your face?

☐ No -->Skip to Question 8

☐ Yes

IF YES

7a) Were you prescribed any treatment for this?

☐ No

☐ Yes

(please specify)

8. Has a doctor ever told you that you have acne?

☐ No -->Skip to Question 9

☐ Yes

IF YES

8a) Were you prescribed any treatment for this?

☐ No

☐ Yes

(please specify)

9. Have you ever tried to become pregnant for 12 months or more without succeeding?

☐ Yes ☐ No

10. Have you ever seen a doctor because you were having trouble becoming pregnant?

☐ No -->Skip to Question 11

☐ Yes

IF YES

10a) Did you have any of the following fertility investigations?

☐ Test of blood or urine hormone levels

☐ Laparoscopy (incision in your stomach to look at your reproductive organs)

☐ Your partner's semen analysed

10b) Did a doctor ever tell you that you or your partner had:

☐ An ovulatory problem?

☐ A tubal problem?

☐ Any other female fertility problem?

- please specify

--

☐ Semen abnormalities?

☐ An unexplained fertility problem?

11. Have you ever been pregnant?

☐ No -->Skip to SECTION E (Page 15)

☐ Yes

12. How many times have you been pregnant?

--	--

 times

13. How many live births have you had?

--	--

13a) When were these babies born?

	Baby	Month	Year
First baby	1		
Second baby	2		
Third baby	3		
Fourth baby	4		
Fifth baby	5		

If you have had more than 5 live births please continue at the end of this section.

14. When you were pregnant were you ever tested for diabetes?

That is, did you have a blood or urine sugar test? This may have involved drinking a very sugary drink.

☐ Yes ☐ No

15. Were you ever told that you had gestational diabetes or pregnancy related diabetes?

☐ Yes ☐ No

--	--	--	--

BEFORE STARTING THIS QUESTIONNAIRE, PLEASE MAKE SURE THAT YOU HAVE READ THE INSTRUCTIONS ON THE PRECEDING PAGES

[illegible]

REMEMBER TO RESET YOUR PEDOMETER EVERY MORNING

7088469675

Women only complete the following section

8 Reproductive History Questions

The following questions are for women only and relate to your reproductive history.

1. What age were you when your periods started?

 Years

2. Between 20 -40 years of age, how many menstrual periods did you **USUALLY** have in a year?

NOTE: EXCLUDE times when you were

- a. Pregnant or breastfeeding.
- b. Taking the oral contraceptive pill

11 or more. ☐ 1

between 6 - 10 ☐ 2

less than 6 ☐ 3

none ☐ 4

If none please state the reason

3. Have you ever been pregnant?

Yes ☐ 1 No ☐ 2

If "NO" proceed to question 7

4. Enter the appropriate number for each of the following.

How many times have you:

A. Been pregnant?

Number of pregnancies

B. Had a miscarriage
/termination?

Number of miscarriages/terminations

C. Given birth to a
child (live or
stillborn)?

Number of live or still births

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5. The next two questions are about breastfeeding.
Have you ever breast fed?

Yes ☐ 1 No ☐ 2

If "NO" proceed to question 7

6. How many children have you breastfed? (Include only those children that you have fed for more than one month)

Number

7. Have you ever used the oral contraceptive pill?

Yes ☐ 1 No ☐ 2

If "NO" proceed to question 9

8. How many years in TOTAL have you ever taken the oral contraceptive pill?
Choose one of the following

Never ☐ 1

Less than one year ☐ 2

1 - 4 years ☐ 3

5 - 10 years ☐ 4

11- 20 years ☐ 5

More than 20 years ☐ 6

9. Have you gone through menopause ("Change of Life")

Yes ☐ 1

No ☐ 2

Don't Know ☐ 3

Currently going through the menopause ☐ 4

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10. Have your periods NOW stopped for more than 12 months?Yes ☐ ¹No ☐ ²Never
had a ☐ ³
period

If "Yes" age when periods stopped

Years

If "No" go to question 14

If "Never" go to question 12

11. Why did your periods stop?Menopause ☐ 1Hysterectomy ☐ 2Don't Know ☐ 3Other ☐ 4

If Other please specify

12. Have you had a hysterectomy?Yes ☐ ¹No ☐ ²

If "Yes" age when had hysterectomy

Years

If "No" go to question 14

2836469671

13. Why did you have a hysterectomy?Endometriosis ☐ 1Prolapsed uterus/urine incontinence ☐ 2Fibroids or cysts ☐ 3Abnormal bleeding ☐ 4Cancer ☐ 5Don't Know ☐ 6Other ☐ 7

If other please specify

--

14. Have you ever had an operation to remove both ovaries?Yes ☐ 1No ☐ 2Don't know ☐ 3

If "No" or don't know go to question 17

15. Age when ovary/ovaries removed?

One or first ovary

Years

--	--

Both or second ovary

Years

--	--

2558469672

16. Why did you have your ovaries removed? (tick all that apply)

Cysts ☐ 1

Endometriosis ☐ 2

Removed at time of hysterectomy ☐ 3

Cancer ☐ 4

Don't Know ☐ 5

Other ☐ 6

If other please specify

17. Are you currently on hormone replacement therapy (HRT)? Yes ☐ 1 No ☐ 2

18. For how many years in TOTAL have you ever used hormone replacement therapy?
(choose one answer only.)

Never used ☐ 1

Less than one year ☐ 2

1 - 4 years ☐ 3

5 - 10 years ☐ 4

More than 10 years ☐ 5

QUESTIONS ABOUT WHAT YOU USUALLY EAT AND DRINK

DAY		MTH	YEAR
		<input type="radio"/> JAN	<input type="radio"/> 2004
		<input type="radio"/> FEB	<input type="radio"/> 2005
<input type="radio"/> 0	<input type="radio"/> 0	<input type="radio"/> MAR	<input type="radio"/> 2006
<input type="radio"/> 1	<input type="radio"/> 1	<input type="radio"/> APR	<input type="radio"/> 2007
<input type="radio"/> 2	<input type="radio"/> 2	<input type="radio"/> MAY	<input type="radio"/> 2008
<input type="radio"/> 3	<input type="radio"/> 3	<input type="radio"/> JUN	<input type="radio"/> 2009
	<input type="radio"/> 4	<input type="radio"/> JUL	<input type="radio"/> 2010
	<input type="radio"/> 5	<input type="radio"/> AUG	<input type="radio"/> 2011
	<input type="radio"/> 6	<input type="radio"/> SEP	<input type="radio"/> 2012
	<input type="radio"/> 7	<input type="radio"/> OCT	<input type="radio"/> 2013
	<input type="radio"/> 8	<input type="radio"/> NOV	<input type="radio"/> 2014
	<input type="radio"/> 9	<input type="radio"/> DEC	<input type="radio"/> 2015

[illegible]

16.

[illegible]

1 can or stubby of beer = 2 glasses
1 large bottle beer (750 ml) = 4 glasses

1 bottle wine (750 ml) = 6 glasses
1 bottle of port or sherry (750 ml) = 12 glasses

17.

[illegible]

18.

MAXIMUM NUMBER OF GLASSES PER 24 HOURS	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19 or more
--	-----	-----	-----	-----	------	-------	-------	-------	-------	------------

□ □



CDAH-Knee Cartilage Study

PHONE QUESTIONNAIRE

Personal Details:

Name:

First Name*

Middle Name(s)

Last Name:

Home address:

Post Code

Postal Address: (if different)

Post Code

Telephone numbers:

Home: Work:

Mobile: Email Address:

Your Date of Birth: / /

1. **(Females only)** Are you currently pregnant? Yes ☐ (Exclude from the study)
No ☐
2. How tall are you? cm OR ft in
3. How much do you weigh? kg OR st lb
4. Which of the following describes your current employment status? You can pick more than one.
- | | |
|----------------------------------|---|
| Working full-time | <input type="checkbox"/> |
| Working part-time | <input type="checkbox"/> |
| Not working (but not retired) | <input type="checkbox"/> |
| Home duties | <input type="checkbox"/> |
| Full-time student | <input type="checkbox"/> |
| Part-time student | <input type="checkbox"/> |
| Retired | <input type="checkbox"/> |
| Permanently unable to work / ill | <input type="checkbox"/> |
| Other | <input type="checkbox"/> (please specify) _____ |
5. Which of the following best describes the occupation you had for the longest period?
- | | |
|--|--|
| Manager or administrator | <input type="checkbox"/> ₁ |
| Professional
(e.g. engineer, doctor, teacher, nurse, police, technical officer) | <input type="checkbox"/> ₂ |
| Tradesperson.....
(e.g. carpenter, electrician, plumber, mechanic etc.) | <input type="checkbox"/> ₃ |
| Clerk
(e.g. typist, receptionist, data processor, book keeper, etc.) | <input type="checkbox"/> ₄ |
| Salesperson or personal service worker
(e.g. sales rep., teller, insurance rep., real estate rep.) | <input type="checkbox"/> ₅ |
| Plant or machine operator, or driver.....
(e.g. taxi driver, bus driver) | <input type="checkbox"/> ₆ |
| Farmer | <input type="checkbox"/> ₇ |
| Labourer or related worker.....
(e.g. trade assistant, factory hand, agricultural labourer, construction, mining) | <input type="checkbox"/> ₈ |
| Member of armed forces | <input type="checkbox"/> ₉ |
| Other, please state..... | <input type="checkbox"/> ₁₀ |
6. Had you had a knee injury requiring non-weight bearing treatment more than 24 hours or surgery during childhood?

Yes ☐No ☐**If 'YES', what type of injury?**

6a) _____

6b) _____

6c) _____

7. Have you had a knee injury requiring non-weight bearing treatment more than 24 hours or surgery in your adult life?

Yes ☐No ☐**If 'YES', what type of injury?**

6a) _____

6b) _____

6c) _____

8. Had you had any knee surgery during your childhood?

Yes ☐No ☐**If 'YES', what type of surgery?**

6a) _____

6b) _____

6c) _____

9. Have you had any knee surgery in your adult life?

Yes ☐No ☐**If 'YES', what type of injury?**

6a) _____

6b) _____

6c) _____

10. Have you had changed your smoking status since YOUR LAST INTERVIEW for this study?

Yes ☐ (Answer Q.11-13 or Q.14)No ☐ **SKIP TO: Q.15**

11. If you started smoking, when did you start smoking daily?

 Years of Age

OR (Year)

12. If you started smoking, how often do you smoke cigarettes, cigars, pipes or any other tobacco products?

Daily

☐

CDAH-2 online questionnaire

Thank you for agreeing to complete this questionnaire for the second follow-up of the Childhood Determinants of Adult Health study (CDAH-2).

This questionnaire will ask you some general information about you and your life, including your physical and emotional health, as well as your medical history.

The questionnaire should take you around 15 minutes to complete. If you get part way through the questionnaire and cannot complete it, please note that you can save what you have already done and come back and complete it later.

There are 62 questions in this survey

Demographic Information

1 [Gender]What is your gender? *

Please choose **only one** of the following:

- ☐ Female
☐ Male

2 [Pregnant]Are you currently pregnant? *

Only answer this question if the following conditions are met:
° Answer was 'Female' at question '1 [Gender]' (What is your gender?)

Please choose **only one** of the following:

- ☐ Yes
☐ No

Only for females.

3 [WeightKg]How much do you weigh in kilograms?

Only answer this question if the following conditions are met:

°

----- Scenario 1 -----

Answer was 'Male' at question '1 [Gender]' (What is your gender?)

----- or Scenario 2 -----

Answer was 'No' at question '2 [Pregnant]' (Are you currently pregnant?)

Please write your answer here:

If you don't know your weight in kilograms, skip this question to record your weight in stones and pounds.

4 [WeightStone]If you don't know your weight in kilograms can you tell me your weight in stone and pounds.

Only answer this question if the following conditions are met:

o

----- Scenario 1 -----

Answer was 'Male' at question '1 [Gender]' (What is your gender?) and Answer was at question '3 [WeightKg]' (How much do you weigh in kilograms?)

----- or Scenario 2 -----

Answer was 'No' at question '2 [Pregnant]' (Are you currently pregnant?) and Answer was at question '3 [WeightKg]' (How much do you weigh in kilograms?)

Please write your answer(s) here:

Stone

Pounds

5 [Employment]Which of the following describes your current employment status? *

Please choose **all** that apply:

- ☐ Working full-time
- ☐ Working part-time
- ☐ Not working (but not retired)
- ☐ Home duties
- ☐ Full-time student
- ☐ Part-time student
- ☐ Retired
- ☐ Permanently unable to work / ill
- ☐ Other:

6 [Education]What is the highest level of education you have completed? *

Please choose **only one** of the following:

- ☐ Primary school
- ☐ Year 7, 8 or 9 or equivalent
- ☐ Year 10 or equivalent
- ☐ Year 12 or equivalent
- ☐ Trade / apprenticeship (eg hairdresser, chef)
- ☐ Certificate / diploma (eg child care, technician)
- ☐ University degree
- ☐ Higher university degree (eg Grad Dip, Masters, PhD)

7 [MaritalStatus]What is your current marital status? *

Please choose **only one** of the following:

- ☐ Single
- ☐ Married
- ☐ De facto
- ☐ Separated / Divorced
- ☐ Widowed
- ☐ Other

8 [Children]How many biological children have you had? *

Please write your answer here:

9 [Child1DOB]In what month and year was the first child born? *

Only answer this question if the following conditions are met:

° Answer was greater than or equal to at question '8 [Children]' (How many biological children have you had?)

Please write your answer(s) here:

Month

Year

10 [Child2DOB] In what month and year was the second child born? *

Only answer this question if the following conditions are met:

° Answer was greater than or equal to at question '8 [Children]' (How many biological children have you had?)

Please write your answer(s) here:

Month

Year

11 [Child3DOB] In what month and year was the third child born?

Only answer this question if the following conditions are met:

° Answer was greater than or equal to at question '8 [Children]' (How many biological children have you had?)

Please write your answer(s) here:

Month

Year

12 [Child4DOB] In what month and year was the fourth child born?

Only answer this question if the following conditions are met:

° Answer was greater than or equal to at question '8 [Children]' (How many biological children have you had?)

Please write your answer(s) here:

Month

Year

13 [Child5DOB] In what month and year was the fifth child born?

Only answer this question if the following conditions are met:

° Answer was greater than or equal to at question '8 [Children]' (How many biological children have you had?)

Please write your answer(s) here:

Month

Year

14 [OtherChildrenDOBs] Please record the month and year of each other birth:

Only answer this question if the following conditions are met:

° Answer was greater than or equal to at question '8 [Children]' (How many biological children have you had?)

Please write your answer here:

15 [Diabetes] Has a doctor or nurse ever told you that you had diabetes? *

Please choose **only one** of the following:

☐ Yes

☐ No

16 [DiabetesYear] In what year were you first told that you had diabetes? *

Only answer this question if the following conditions are met:

° Answer was 'Yes' at question '15 [Diabetes]' (Has a doctor or nurse ever told you that you had diabetes?)

Please write your answer here:

The Appendices 14, 16 have been removed due to copyright or proprietary reasons.

Appendices 13, 14 and 17 are the published versions of chapters 4, 5 and 8 respectively.

Appendix 16 is a stand alone published article not found in the main body of the thesis.

Wei et al. *BMC Musculoskeletal Disorders* 2010, **11**:288
<http://www.biomedcentral.com/1471-2474/11/288>



RESEARCH ARTICLE

Open Access

Menstrual irregularity and bone mass in premenopausal women: Cross-sectional associations with testosterone and SHBG

Shuying Wei^{1*}, Graeme Jones¹, Russell Thomson¹, Petr Otahal¹, Terry Dwyer², Alison Venn¹

Abstract

Background: There have been few studies examining the associations between menstrual irregularity, androgens and bone mass in population-based samples of premenopausal women. This study aimed to describe the associations between menstrual pattern, testosterone, sex hormone binding globulin (SHBG) and bone mass in a population-based sample of premenopausal women.

Methods: Cross-sectional study (N = 382, mean age 31.5 years). Menstrual pattern was assessed by questionnaire, bone mass measured by quantitative ultrasound (QUS) and androgen status was assessed by levels of serum testosterone, SHBG and the free androgen index (FAI).

Results: Women with irregular cycles (n = 41, 11%) had higher free androgen index (FAI, P = 0.01) and higher QUS measurements including speed of sound (SOS, 1%, P < 0.05), quantitative ultrasound index (QUI, 7%, p < 0.05), and broadband ultrasound attenuation (BUA, 7%, p = 0.10). These associations persisted after adjustment for age and body mass index (BMI). After further adjustment for hormonal factors (either testosterone, SHBG or FAI), the strength of the associations was moderately attenuated, however, women with irregular cycles still had a 6% increase in mean QUS. Total testosterone, FAI and SHBG were also associated with QUS measures (testosterone and FAI, r +0.11 to +0.21, all p < 0.05; SHBG r -0.14 to -0.16, all p < 0.05) and the associations remained significant after adjustment.

Conclusion: Irregular menstrual cycles were associated with higher bone mass in this population-based sample of premenopausal women suggesting menstrual disturbance should continue to be evaluated but may be less harmful for bone mass. The association between menstrual irregularity and bone mass was partially mediated by markers of androgen status especially free testosterone.

Background

Osteoporosis is a major public health concern [1]. Most osteoporosis sufferers are postmenopausal women and age-related estrogen deficiency is considered a major cause of bone loss [2]. As well as estrogen, androgens may also have an effect on bone metabolism in women; excess levels of androgen are associated with higher bone mass in women with Polycystic Ovary Syndrome (PCOS) [3-7]. Menstrual irregularity has been associated with lower bone mass in studies of female athletes who had low BMI and extensive training [8-11]. However

these results may not directly apply to today's population of young women with a higher prevalence of overweight and obesity. Recent studies have suggested that menstrual irregularity is associated with higher levels of androgens in a population-based sample of premenopausal women [12,13], while in female elite athletes oligomenorrhea was associated with higher bone mass and PCOS [14]. These results may in turn suggest a positive association between menstrual irregularity and bone mass. However, few studies have examined the associations between menstrual irregularity, androgen and bone mass in population-based sample. Therefore, the aim of this cross-sectional study was to describe the association between menstrual pattern, testosterone, sex hormone binding globulin (SHBG) and bone mass measured by

* Correspondence: weis@utas.edu.au

¹Menzies Research Institute, University of Tasmania, Hobart, Australia
Full list of author information is available at the end of the article



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quantitative ultrasound (QUS) in a population-based sample of young women.

Methods

Subjects

This study utilized data from the Childhood Determinants of Adult Health (CDAH) study, a 20-year follow-up of children who were randomly selected for the 1985 Australian Schools Health and Fitness Survey (ASHFS) at age 7-15 years. Details of the 1985 sampling strategy have been described elsewhere [15]. During follow-up (2004-2006) a total of 2410 subjects aged 26-36 years (48% male, 52% female), completed questionnaires and attended one of 34 study clinics in major cities and regional centres around Australia for extensive physical measurements including anthropometric measures, quantitative ultrasound and blood biochemistry.

Eligible subjects for this study were women who had completed study questionnaires, attended study clinics, were not currently taking oral contraceptives, and not pregnant or breast feeding at the time of clinic attendance. Of the 1260 women who attended clinics, 82 were currently pregnant; 453 were currently taking hormonal contraceptives (including combined and progestin only contraceptives and progestin releasing intrauterine devices) or medications such as hormonal agents for PCOS or in vitro fertilization (IVF); 44 did not provide their menstrual cycle characteristics; and 299 had missing QUS measurements due to the machine being out of service, leaving a total of 382 participants for this analysis. All participants provided written informed consent and the study was approved by the Southern Tasmania Health and Medical Human Research Ethics Committee.

Assessment of menstrual cycle characteristics

Menstrual cycle characteristics were obtained by written questionnaire. We defined the menstrual cycle as the time from the first day of one period to the first day of the next. The question was then asked: "thinking about the most recent time when you were having periods and were not using hormonal contraceptives (e.g., the pill) and were not pregnant or breast feeding: would you describe your period as very regular, fairly regular, irregular or very irregular [16]. To simplify analyses, menstrual cycle pattern was defined as regular if women described their periods as very or fairly regular, and irregular if irregular or very irregular.

Hormone measurements

Blood samples (32 ml) were collected from participants after an overnight fast. Plasma insulin was initially measured by a microparticle enzyme immunoassay kit (AxSYM; Abbot Laboratories, Abbot Park, IL) and later,

following a change in the choice of kit by the testing laboratory, by electrochemiluminescence immunoassay (Elecsys Modular Analytics E170; Roche Diagnostics, Mannheim, Switzerland). Due to this change in assay methodology, insulin levels from participants' samples ($N = 258$) assayed using the first methodology were corrected to levels in samples assayed using the second methodology (as per correction factor equation of the laboratory). Total testosterone concentrations were estimated by radioimmunoassay (RIA) developed by Repromed Laboratory (Dulwich, South Australia), which is sensitive for lower levels of testosterone down to 347 pmol/L. SHBG was measured using a non-competitive liquid-phase immunoradiometric assay (SHBG-IRMA kit, Orion Diagnostica, Espoo, Finland). For testosterone, the intra- and inter- assay coefficients of variation (CV) were 6% at 1 nmol/l and 15%, respectively. For SHBG, the intra- and inter- assay CV were 15.4%, and 2.0-8.6% respectively. FAI was calculated as: testosterone (nmol/L) * 100/SHBG (nmol/L).

Anthropometric measurements

Anthropometric measures were taken at study clinics by trained personnel. Height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively, and body mass index (BMI) was calculated as the ratio of weight (kg) to height (m) squared (kg/m^2). Waist to hip ratio (WHR) was calculated by dividing waist by hip circumference measured to the nearest 0.1 cm.

Quantitative ultrasound measurements

All participants had calcaneal QUS measured by a single Sahara Clinical Bone Sonometer (Hologic Inc., MA, USA). The ultrasound system consists of two sound transducers mounted coaxially on a motorized calliper with one transducer acts as an emitter and the other as a receiver. This makes direct contact with the heel through elastomer pads and an ultrasonic coupling gel. The lower part of the dominant leg was placed immobilized during measurement and the proper leg angle set by a positioning aid. Broadband ultrasound attenuation (BUA, dB/MHz) and speed of sound (SOS, m/s) were measured at a fixed region in the mid-calcaneus. The quantitative ultrasound index (QUI) was derived from SOS and BUA using the equation: $\text{QUI} = 0.41 \times (\text{BUA} + \text{SOS}) - 571$ [17]. Quality assurance was performed daily by calibrating the device on a dedicated phantom supplied by the manufacturer. The coefficient of variation (CV) for QUS measures was 1%.

Assessment of other covariates

Demographic and lifestyle information was obtained by questionnaire. Smoking status was classified as non-smoker or current smoker. Women were classified as

nulliparous if they had not had a live birth or parous if they had had at least one live birth. Physical activity was assessed as we have described previously [18] by the International Physical Activity Questionnaire (IPAQ) and as mean steps per day measured by Yamaz pedometer worn for seven days. The questionnaire asked whether participants had ever been told by a doctor that they had polycystic ovaries (PCO) or polycystic ovary syndrome (PCOS).

Statistical Analysis

The unequal variance t-test was used to assess differences in normally-distributed continuous characteristics in this study across categorized menstrual cycle pattern (regular and irregular), the Wilcoxon rank-sum test was used for skewed continuous variables and the two-sample test of proportions was used for dichotomised covariates. Spearman correlations were used to estimate crude associations between continuous exposures and QUS. Multivariable linear regression analysis was used to explore the association between all QUS measures and menstrual cycle pattern and hormonal factors (testosterone, SHBG and FAI). QUS parameters and hormonal factors were logarithmically transformed to fit linear regression models and standardised to enable direct comparisons between BUA, SOS and QUI. The coefficients represent a change of one standard deviation. Age, BMI and smoking were considered as confounders or covariates of importance, and adjusted for in multivariable models where appropriate. All statistical analyses were performed on intercooled Stata 9.2 for windows (Statacorp, Texas, USA).

Results

In this relatively young study sample with age range of 26–36 years (mean age 32 years; mean BMI 24.8 kg/m²), most women were married or living as married (61.7%) and were non-smokers (85.7%). Over half the women had had at least one livebirth (53.7%). Only 11% of participants had irregular menstrual cycles. Very few women sampled reported having ever been told by a doctor that they had PCO or PCOS (n = 9 with irregular cycles, n = 9 with regular cycles). Women currently taking hormonal medication for PCOS (n = 2) were ineligible for this analysis and excluded. Compared with other women in this study, women who were excluded due to current use of hormonal contraceptives or hormonal medications, were slightly younger (30.7 vs 31.5 years) and more likely to have a history of menstrual irregularity (21.6% vs 10.7%), but there was no significant difference in BMI between the groups (25.0 kg/m² vs 24.8 kg/m², p = 0.54) and similar proportions were current smokers (13.2% vs 14.3%, p = 0.69).

In the unadjusted analysis, women with irregular cycles had higher FAI, SOS and QUI, and lower SHBG compared with those with regular cycles (Table 1). The difference in bone mass between women with regular and irregular cycles is illustrated in Figure 1. There were no significant differences in age, body composition measurements, marital status, number of live births, smoking and plasma insulin levels between women with irregular cycles and regular cycles. However, all anthropometric measures including BMI, waist circumference and waist to hip ratio were significantly associated with BUA (p < 0.05–0.01) (Table 2) as were hormonal factors with a positive association between testosterone and FAI, and a negative association with SHBG (all p < 0.01) (data not shown).

There were significant associations between hormonal factors and BUA, SOS and QUI measurements found in both unadjusted correlation analysis (Table 2) and adjusted analysis (Table 3). Total testosterone and

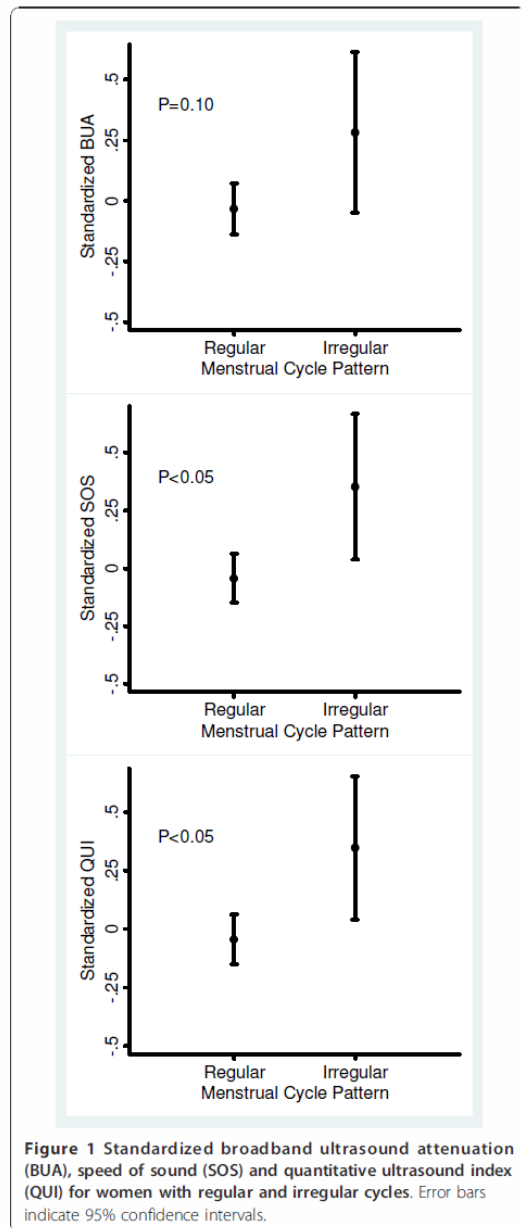
Table 1 Characteristics of participants by menstrual cycle pattern

Characteristics	Regular cycle N = 341	Irregular cycle N = 41	P values
Age (year)^a	31.6 (2.6)	30.9 (2.4)	0.11
Anthropometrics^a			
Body mass index (kg/m ²)	24.8 (5.0)	24.5 (5.9)	0.77
Waist circumference (cm)	78.8 (11.4)	77.8 (13.3)	0.65
Waist-hip ratio	0.757(0.062)	0.760 (0.061)	0.75
Married^b %	62	56	0.44
Parous^b %	54	49	0.50
Current smoker^b %	15	13	0.73
Physical activity (PA)^c			
Pedometer steps (per day)	8331 (6758-10623)	8325 (6715-10992)	0.68
Leisure time PA (min/week)	120 (0-219)	66 (0-180)	0.24
Total PA (min/week)	680 (385-1030)	560 (300-970)	0.27
Hormonal factors^c			
Testosterone (nmol/L)	1.50 (1.22-1.86)	1.53 (1.35-1.89)	0.24
SHBG (nmol/L)	50.3 (35.8-68.0)	43.6 (30.1-54.6)	0.01
Free androgen index	2.94 (2.00-4.47)	4.22 (2.60-6.24)	0.01
Insulin (mU/l)	3.9 (5.3-7.5)	3.5 (4.9-7.9)	0.85
Quantitative ultrasound^c			
BUA (dB/MHz)	73.6 (65.4-84.6)	78.7 (67.9-87.1)	0.10
SOS (m/s)	1563 (1543-1587)	1569 (1557-1606)	0.02
QUI (%)	99.5 (88.6-114.1)	106.7 (94.6-121.6)	0.03

^a presented by mean and standard deviation (SD), P values derived from unequal variance t-test.

^b presented by percentage, P values derived from test of proportions.

^c presented by median (inter-quarter range), P values from Wilcoxon rank-sum test. Bold denotes significant results.



FAI were significantly positively associated with QUS measurements, while SHBG was negatively associated with QUS measures. These associations remained significant after adjustment for confounders with the exception of SHBG and BUA. Further adjustment for history of PCO/PCOS did not change the significant

Table 2 Spearman correlation coefficients (r) for associations of quantitative ultrasound measures and continuous variables in the study

Characteristics	BUA		SOS		QUI	
	r	P	r	P	r	P
Anthropometrics						
Body mass index	0.17	< 0.01	-0.01	0.79	0.05	0.35
Waist circumference	0.17	< 0.01	-0.01	0.86	0.05	0.32
Waist-hip-ratio	0.14	0.01	0.03	0.57	0.07	0.16
Hormonal factors						
Testosterone	0.14	0.01	0.09	0.08	0.11	0.04
SHBG	-0.16	< 0.01	-0.14	0.01	-0.16	< 0.01
Free androgen index	0.21	< 0.001	0.16	< 0.01	0.19	< 0.001

SHBG = sex hormone-binding globulin. BUA = broadband ultrasound attenuation. SOS = speed of sound. QUI = quantitative ultrasound index. Bold denotes significant association ($P < 0.05$).

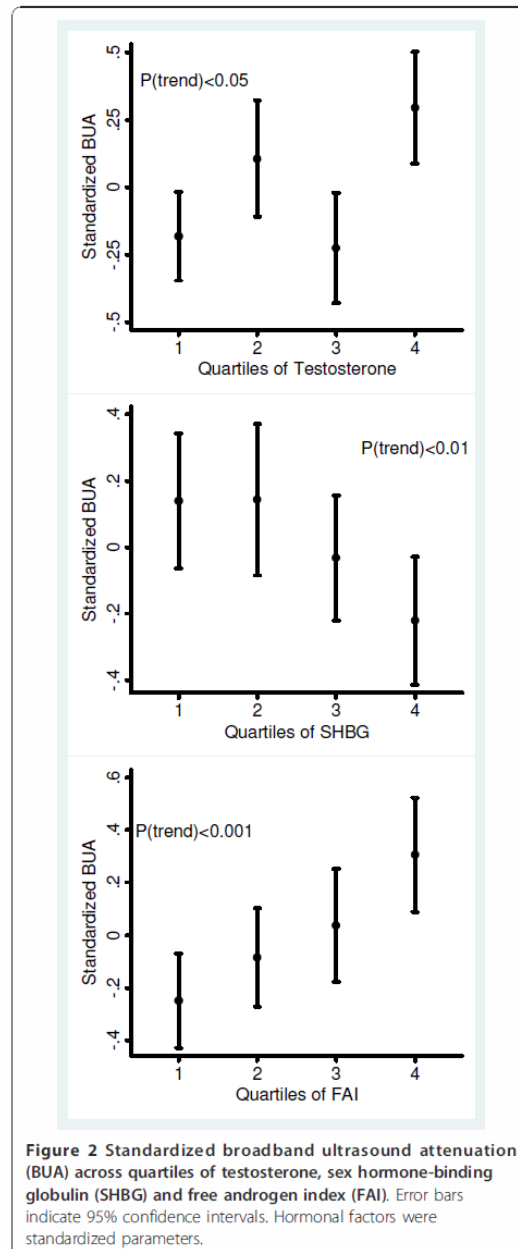
associations. The association between hormonal factors and QUS measures is further illustrated in Figure 2 where there were dose-response relationships between BUA measures and hormonal factors, especially for FAI.

The associations between irregular cycles and QUS measures are presented in Table 4. In multivariable regression analysis, irregular cycles remained significant associations with SOS and QUI ($\beta = 0.38$ for both, $p < 0.05$), with a consistent trend for BUA ($\beta = 0.31$, $p = 0.06$) after adjustment for confounders. The strength of

Table 3 Association between quantitative ultrasound (QUS) parameters and hormonal factors

	Unadjusted model	Adjusted model	
	(n = 382) β (95% CI)	Model 1 (n = 358) β (95% CI)	Model 2 (n = 358) β (95% CI)
BUA			
Testosterone	0.15 (0.04, 0.24)	0.14 (0.04, 0.25)	0.13 (0.03, 0.24)
SHBG	-0.14 (-0.24, -0.04)	-0.07 (-0.19, 0.05)	-0.05 (-0.17, 0.06)
FAI	0.18 (0.08, 0.28)	0.14 (0.03, 0.26)	0.13 (0.01, 0.24)
SOS			
Testosterone	0.11 (0.01, 0.21)	0.13 (0.03, 0.24)	0.12 (0.02, 0.23)
SHBG	-0.13 (-0.23, -0.03)	-0.16 (-0.27, -0.04)	-0.14 (-0.26, -0.02)
FAI	0.16 (0.06, 0.26)	0.20 (0.08, 0.31)	0.18 (0.06, 0.29)
QUI			
Testosterone	0.12 (0.02, 0.22)	0.14 (0.03, 0.24)	0.13 (0.02, 0.23)
SHBG	-0.14 (-0.24, -0.04)	-0.13 (-0.25, -0.01)	-0.11 (-0.23, 0.01)
FAI	0.17 (0.07, 0.27)	0.18 (0.07, 0.29)	0.16 (0.05, 0.28)

BUA = broadband ultrasound attenuation. SOS = speed of sound. QUI = quantitative ultrasound index. SHBG = sex hormone-binding globulin. FAI = free androgen index. QUS measures and hormonal parameters were log transformed before converted into standardized variables. β = standardised beta coefficients. Model 1 adjusted for age, BMI and smoking. Model 2 further adjusted for menstrual cycle pattern. Bold denotes significant association ($P < 0.05$).



the associations was moderately attenuated when further adjusted for hormonal factors (testosterone, SHBG and FAI separately), with maximally a 27% decrease in the coefficient when further adjusted for FAI, and a lesser effect when adjusted for total levels of testosterone.

However women with irregular cycles still had an increase of 0.30-0.31 in the standardized log-transformed SOS and QUI and this corresponds to a maximum 6% increase in the mean QUS measurements. We recalculated free testosterone (FT) in the equation by taking into account the laws of mass action and using a stable mean of albumin concentration (assumed to be 43 g/l), and found that the associations between menstrual irregularity and bone mass were nearly the same when adjusted for FAI and FT. Further adjustment for a history of ever been told by a doctor that they had PCO or PCOS made the associations a little stronger. When irregular and very irregular cycles were examined separately, the adjusted associations were similar in magnitude but were not statistically significant, likely due to lower power ($n = 22$ for irregular, $n = 19$ for very irregular; data not shown).

Discussion

In this cross-sectional study of a population-based sample of premenopausal women (37% overweight or obese), an irregular menstrual cycle was associated with higher quantitative ultrasound measurements including BUA, SOS and QUI. This association was independent of age and BMI but was moderately reduced when further adjusted for hormonal factors, particularly for SHBG and FAI, suggesting that these hormonal factors partially mediate the associations.

Studies have reported a negative association between oligo/amenorrhea and bone mineral density [8-11,19] in female athletes with low BMI. The bone loss was attributed to low levels of estrogen and/or chronic energy deficiency caused by restricted food intake and extensive training [20]. However, these conditions are not common in the general community and these results may not be applicable to young women affected by today's obesity epidemic. In fact, in this population-based sample of young women, the mean BMI was 24.8 kg/m^2 which is close to the lower cut-point of overweight ($25-30 \text{ kg/m}^2$) based on the WHO categorization [21]. We found that irregular cycles were associated with higher bone mass and this was partially mediated by SHBG and FAI. This is consistent with other studies' findings that bone mass was associated with higher levels of androgens and lower levels of SHBG [22-24]. Furthermore we found a significant dose-response relationship between hormonal factors and bone mass, especially for FAI.

Few studies have examined bone mass and the association with menstrual irregularity and androgens in population-based samples of premenopausal women though positive associations between amenorrhea and bone mass were reported in women with hirsutism and PCOS - conditions with excess androgen. Endogenous

Table 4 Association between quantitative ultrasound (QUS) measures and menstrual irregularity adjusted by each hormonal factor separately

	Adjusted model * β (95% CI)	Adjusted model + testosterone β (95% CI)	Adjusted model + SHBG β (95% CI)	Adjusted model +FAI β (95% CI)
BUA				
Regular cycle	Ref	Ref	Ref	Ref
Irregular cycle	0.31 (-0.01, 0.64)	0.29 (-0.04, 0.61)	0.28 (-0.05, 0.61)	0.25 (-0.07, 0.58)
SOS				
Regular cycle	Ref	Ref	Ref	Ref
Irregular cycle	0.38 (0.05, 0.71)	0.36 (0.03, 0.68)	0.31 (-0.02, 0.64)	0.30 (-0.03, 0.63)
QUI				
Regular cycle	Ref	Ref	Ref	Ref
Irregular cycle	0.38 (0.05, 0.71)	0.36 (0.03, 0.68)	0.32 (-0.01, 0.65)	0.31 (-0.02, 0.63)

BUA = broadband ultrasound attenuation. SOS = speed of sound. QUI = quantitative ultrasound index. SHBG = sex hormone-binding globulin. FAI = free androgen index. QUS measures were log transformed before converted into standardized values. β = standardised beta coefficients. *Adjusted model controlled for age and BMI. Bolder denotes significant association ($P < 0.05$).

androgens were associated with menstrual irregularity in population-based [12,13] and clinical samples [25], and bone mass in premenopausal women [22,23,26], but these associations were investigated separately. An important strength of our study, therefore, was its population-based sample and ability to examine the role of hormonal factors.

The mechanism for the association between menstrual irregularity, hormonal factors and bone mass is not clear. However the association between menstrual irregularity and bone mass had a moderate reduction in strength and lost significance when further adjusted for either SHBG or FAI separately. This suggests that bone mass could be influenced by SHBG through the regulation of free testosterone levels. Our result is consistent with a prospective study which examined 231 women aged 32-77 years over 2-8 years period and found, in addition to age and weight, SHBG was a strong independent predictor of BMD [26]. A recent editorial discussed a potential dual role for SHBG (inhibitor or facilitator) in regulating sex hormone action [27] following a genetic study of polymorphisms in the SHBG gene promoter which found that serum levels of SHBG were positively associated with BMD in elderly men [28]. However our result favours the free hormone hypothesis that SHBG inhibits sex hormone bioavailability, rather than augments sex hormone action.

There were several limitations which should be considered when interpreting these findings. First, menstrual cycle characteristics were obtained by self-administered questionnaire which did not include specific criteria for women to assess their cycle regularity. This may have resulted in misclassification. However, it is unlikely that this error would be differential with respect to bone mass or hormonal factors since both QUS and hormonal factors were measured at the same

time that women reported their menstrual cycles. Therefore the association between irregular cycles and bone mass may be underestimated in our study. Second, we did not have dual-energy x-ray absorptiometry (DXA) measurement of BMD; however QUS results correlate well with BMD measured by DXA at the heel [29] and predict fracture risk similarly to DXA [30]. This study was conducted in community centres across Australia so DXA measurements were not feasible. Furthermore, use of a portable QUS machine avoided standardisation issues which could have been a major problem had we used multiple DXA instruments at different sites. Third, we did not assess endogenous estrogens which show substantial variation during the menstrual cycle. Clearly, estrogens play a major role in regulating bone mass in both males and females. Previous studies examining both estrogens and androgens in premenopausal women found that androgen but not estrogen was associated with bone mass [22,26] suggesting premenopausal estrogen levels may be sufficient in most women. Due to logistic issues, the blood sample collection was not timed with the menstrual cycle possibly introducing some measurement error as testosterone varies with the menstrual cycle [31]. Another limitation is that some young women with irregular cycles may have had undiagnosed PCOS. However, this sample of young women had testosterone levels typically within the normal range. Exclusion of eight women who had testosterone levels higher than 2.9 nmol/l (the upper limit of the laboratory's reference range for premenopausal women with normal menstrual cycles and no evidence of polycystic ovaries on ultrasound) or 24 women who had SHBG levels less than 24 nmol/l (the lowest levels for follicular phase for premenopausal women) made little difference to the findings. In addition, adjustment for a history of ever being told by a doctor they had PCO or

PCOS did not decrease the associations. Further, the relatively low percentage of eligible participants with complete data may limit the generalizability of our findings to the general population. Finally, we cannot be certain of the causal direction of the associations observed due to the cross-sectional design of this study. Thus, a longitudinal study is necessary to confirm these findings.

Conclusions

Irregular menstrual cycles were associated with higher bone mass in this population-based sample of premenopausal women suggesting menstrual disturbance should continue to be evaluated but may be less harmful for bone mass than previously believed. The association between menstrual irregularity and bone mass was partially mediated by markers of androgen status especially free testosterone.

Acknowledgements

We thank the CDAH project manager Marita Dalton, all project staff and study participants for their valuable contributions to this study. The study was funded by the National Health and Medical Research Council, National Heart Foundation, Tasmanian Community Fund and Veolia Environmental Services.

Author details

¹Menzies Research Institute, University of Tasmania, Hobart, Australia.
²Murdoch Children's Research Institute, Victoria, Australia.

Authors' contributions

SW contributed to the design of the study, performed the statistical analysis and drafted the manuscript. AV, GJ and TD contributed to the study design, data collection and revised the manuscript critically. RT and PO participated in analysis and interpretation of data. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 21 June 2010 Accepted: 29 December 2010
 Published: 29 December 2010

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Pre-publication history

The pre-publication history for this paper can be accessed here:
<http://www.biomedcentral.com/1471-2474/11/288/prepub>

doi:10.1186/1471-2474-11-288

Cite this article as: Wei et al.: Menstrual irregularity and bone mass in premenopausal women: Cross-sectional associations with testosterone and SHBG. *BMC Musculoskeletal Disorders* 2010 **11**:288.